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CONTENTS

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Patterns of Esterases During the Postnatal Development of Wing Polymorphism in *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae)

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Abstract: The quantitative and qualitative estimations of the esterases activity were made during the postnatal development of wing polymorphism in *Lipaphis erysimi* (Kalt.). Two peaks of activity during the development of alatae and three in apterae were observed and the maximum activity was in the Ist instar nymphs and minimum in the alatae adult. The number of esterases isozymes varied from 4-8 in different developmental stages. The appearance of additional isozymes has been related to specific functional requirements of the respective stage/form.

Keywords: esterases, wing polymorphism, electrophoresis, development, *Lipaphis erysimi* (Kait).

INTRODUCTION

The members of the family Aphididae have two special features i. e., the presence of parthenogenesis and wing polymorphism. Both of these features help them to survive in ephemeral habitats and to acquire the status of major pests of many crops. The wing polymorphism involves the development of alatae under the influence of certain intrinsic and extrinsic factors (Hardie and Lees, 1985) and thus helps in migration. Although karyologically there is no difference in various wing morphs of aphids (Chattopadhyay and Raychaudhuri 1980) metabolically it could be hypothesized that the enzyme activities might be varying during their postnatal development due to the specific necessities of the respective form. The esterases constitute a major group of hydrolytic enzymes and have been reported to show specificity during development in some of the insects, quantitatively (Aronshtam and Kuzin, 1974) as well as qualitatively (Yoo 1979; Lu *et al.*, 1988) and this specificity was related to their involvement in moulting and metamorphosis. The extent of implication of esterases in the wing morph development could be ascertained only after making quantitative estimations and by studying the isozyme patterns during development. This is the theme of the present study. The mustard aphid, *Lipaphis erysimi* (Kalt.) was selected as the model aphid because it is a major pest of Brassica crops in India and its life cycle comprises both alatae and apterae morphs.

MATERIALS AND METHODS

The radish *(Raphanus sativus L.)* was used as host plant for rearing *L. erysimi*. Potted plants were used for the rearings under 10L: 14D regime at 20±2°C in caged conditions. The alatae and apterae nymphs were differentiated on the basis of presence or absence of wing buds and the adults were identified on the basis of presence or absence of wing. The freshly emerged aphids (50-100 per pot) were released on 4-5 pots for giving birth to nymphs for 6-8 hours and the nymphs required for first, second, third and fourth instars and adults were collected after 24, 48, 102, 158 and 218 hours, respectively.

The extraction of esterases from nymphs of first, second, third (Apterae and Alatae), fourth (AP and AL) instars and adults (AP and AL) was done separately by following th method of Katzenellenbogen and Kafatos (1971). The homogenates 0.1% and 0.4% of aphids prepared in 0.1 M phosphate buffer (pH 6.5) were centrifuged at 10,000 rpm for quantitative and qualitative analysis, respectively. The procedure of Katzenellenbogen and Kafatos (1971)

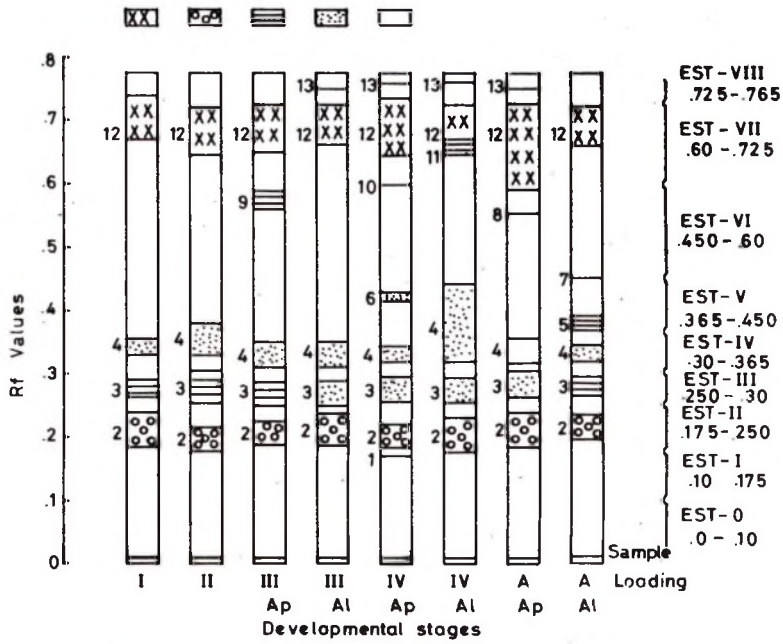

Fig. 1 Zymogram of Estrases during the development of polymorphic forms of L. erysimi.

was followed for quantitative estimations of esterases. The PAGE (polyacrylamide gel electrophoresis) was performed for qualitative analysis by using the methods of Davis (1964) and Ornstein (1964). The isozymes of esterases were classified according to their decreasing staining intensities (++++, +++, ++, and trace) and they were also numerically ordered in a cathodal to anodal sequence. All of the experiments were repeated at least three times for each nymphal instar and adults, both for quantitative as well as qualitative analyses.

Results and Discussion

The quantitative estimations for the esterases activity revealed that the overall activity was at a lower plataeu in the alatae compared to the apterae form during development and the lowest activity was recorded in the adult alatae. There were two peaks of activity (in Ist and 3rd instar) during the development of alatae morphs and three (in Ist and 3rd instar and adults) in apterae (Table 1).

Table 1. Activity of total esterases in the postnatal development of polyphenics of *L. erysimi*

Nymphal instar/ Adult	Esterase activity (UM/25mg body wt.)	
	Alatae (Mean±SD)	Apterae (Mean±SD)
I instar	80.91+2.032	80.91+2.032
II	68.22+3.189	68.22+3.189
III	77.59+3.658	78.73+4.124
IV	41.09+1.626	56.25+3.542
Adult	37.64+1.702	73.73+2.085

The sexual dimorphism in the esterases activity has been demonstrated in *Drosophila melanogaster, D. simulans, Locusta migratoria migra*

Table 2. Rh values of exterase isozymes in the apterae and alatae of *L.erysimi* in the course of postnatal development

Instar/Adult	Isozyme number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
I	-	0.210	0.276	0.342	-	-	-	-	-	-	-	0.704	-
II	-	0.196	0.285	0.348	-	-	-	-	-	-	-	-	-
III apeterae	-	0.206	0.269	0.331	-	-	-	-	0.575	-	-	0.678	-
III apterae	-	0.212	0.269	0.331	-	-	-	-	-	-	-	0.694	0.750
IV apterae	0.171	0.201	0.274	0.329	-	0.421	-	-	-	0.598	-	0.689	0.756
IV alatae	-	0.202	0.272	0.329	-	-	-	-	-	-	0.652	0.696	0.759
Adult apterae	-	0.210	0.283	0.335	-	-	-	0.553	-	-	-	0.658	0.750
Adult alatae	-	0.213	0.274	0.329	0.378	-	0.451	-	-	-	-	0.689	-

Table 3. Distribution and intensities of esterase isozymes in postnatal development of polymorphism in *L.erysimi*

Instar/Adult	Total number of isozymes	Intensity of isozymes				
		++++	+++	++	+	Trace
I	4	$1(12)$$	1(2)	1(3)	1(4)	-
II	4	1(12)	1(2)	1(3)	1(4)	-
III apterae	5	1(12)	1(2)	2(3,9)	1(4)	-
III alatae	5	1(12)	1(2)	-	2(3,4)	1(3)
IV apterae	8	1(12)	1(2)	-	3(3,4,6)	3(1,10,13)
IV alatae	6	1(12)	1(2)	1(11)	2(3,4)	1(13)
Adult apterae	6	1(12)	1(2)	-	1(3)	3(4,8,13)
Adult alatae	6	1(12)	1(2)	2(3,5)	1(4)	1(7)

$ Number of isozymes; $$ Serial number of isozymes

torioides and *Callosobruchus maculatus* by Afonoshtan and Kuzin (1974) Curry (1977) and Sharma and Sharma (1981), respectively. The high levels of esterases activity has been even reported in the nomadic phase of Nearctic army ant, *Neivamyrmex nigrescens* (Wang and Happ, 1974). The high activity observed in the first instar nymphs of both the morphs could be associated with the metabolic changes taking place in the early differentiation of two morphs. The apterous adults have been observed to be much more fecund compared to the alatae and therefore require higher rates of vitellogenesis, which might be the cause for the third peak in the apterous adults.

In the PAGE analysis of esterases, eight distinct Zones (Est I-VIII) of activity could be detected in the mustard aphid, and these were represented by thirteen isozymes with a minimum of four and a maximum of eight isozymes in any single developmental stage (Table 2 and 3; Fig. 1).

On the basis of their appearance these isozymes could be divided into two categories. the first group constituted by isozyme no. 2,3.4 and 12 persisted throughout the development of both the morphs. The size and intensities of these isozymes especially no. 2 and 12 revealed the same pattern as was found in the quantitative analysis of two morphs. The second group included isozyme no. 1, 5, 6, 7, 8, 9, 10, 11 and 13 which appeared or disappeared in different developmental stages of both the morphs; moreover, these isozymes were of lower intensities and of smaller sizes. Both of these features indicated that these

isozymes appeared in small quantities for specific purposes. Among aphids, variable number (2.7) of isozymes have been reported in different species. Kharsu, *et al.* 1972; Brestkin *et al.* 1985; Brookes and Loxdale, 1987). Castenera *et al.* (1985) failed to find any specific modulations in esterase isozyme patterns of apterae and alatae from first to fourth instar nymphs in wheat aphid, *Sitobion avenae*. However, a function related variation in the number of isozymes of esterases have been documented in the females of *C. maculatus* and males of *Drosophila dunn* by Sharma and Sharma (1981) and Carrasco et al. (1984), respectively.

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ENTOMON 1995 VOL. 20 NO. 3 & 4: 169-173

Impact of Varying Biochemical Profiles of *Ricinus Communis* Linn. on the Haemodynamics of *Pericallia ricini* Fabr. (Arctiidae: Lepidoptera)

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Abstract: Variation in the nutrient quality of two castor varieties shows a differential impact on haemolymph biochemistry and haemocyte count of the larvae of *Pericallia ricini*. Individuals fed on red stemmed castor showed maximum amount of primary metabolites in their haemolymph whereas individuals fed on green stemmed castor showed greater haemocyte diversity. Implications on such biochemical changes on total haemocyte count (THC) and differential haemocyte count (DHC) are also discussed.

Key words: *Pericallia ricini*, castor, haemolymph biochemistry, haemocyte count.

INTRODUCTION

Insect haemolymph serves as a repository for stored nutrients, besides those continuously derived from digestion of ingested food, essentially comprising primary and secondary metabolites. The nutritional quality of host plants tend to influence the haemolymph composition of insects (Bhatt and Krishna, 1984). It is well known that haemocyte counts vary with the physiological condition of insects. Though haemocyte numbers per unit volume often increase in immature insects from one instar to the next (Arnold and Hinks, 1976; Wago and Ishikawa, 1979), there is a slight decrease in the total number of haemocytes per unit volume particularly just before ecdysis (Pathak, 1986), resulting in a fluctuation in the slow increase in haemocyte numbers among the developing immatures. While there is a decrease in the pupal haemocyte counts, the numbers are known to increase in the young adult, but not reaching the condition seen in the immature. Changes in the haemocyte population with regard to the types during development, is seen in many species (Gupta, 1985; Vinson, 1993). The impact of plant chemicals on the chemical composition of the haemolymph, as well as on the haemocyte diversity, is an aspect deserving greater attention. As such this study aims at assessing the changes in the biochemical profiles of the haemolymph and haemocyte count of the larvae of *Pericallia ricini*, a highly polyphagous lepidopteran pest of economically important agricultural and horticultural crops, in relation to the biochemistry of the leaves of red and green stemmed varieties of castor, *Ricinus communis*.

MATERIALS AND METHODS

A standard laboratory culture of *P. ricini*, reared separately on fresh leaves of green and red stemmed castor plants, served as the source of larvae for this investigation. Third, fourth and fifth instars of *P. ricini* were selected for the haemolymph studies. The prolegs of these instars were severed with fine scissors and 10 µl haemolymph was collected from each instar in a clean test tube. Estimations of primary and secondary metabolites of fresh leaves of castor and of the haemolymph of the larvae were carried out following the methods of Lowry *et al.*, (1951) for total proteins, Moore and Stein (1948) for total amino acids; Dubois *et al.*, (1956) for carbohydrates; Folch *et al.*, (1957) for total lipids; Bray and Thrope (1954) for total phenols.

For ascertaining differences in total and differential haemocyte count, the technique of Gupta (1979) was used. Haemolymph collected

Fig.1 Differential haemocyte count of *Pericallia ricini* reared on red and green stemmed castor leaves

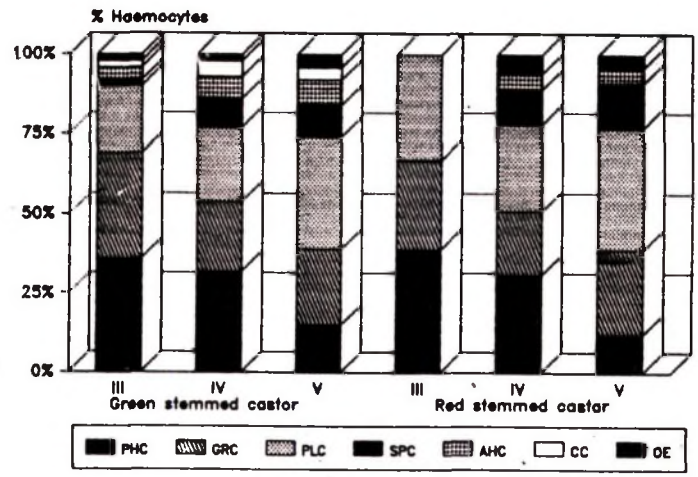

Table 1
Biochemical profiles of two varieties of castor leaves

Varieties	Total Carbohydrates (mg/g)	Total Proteins (mg/g)	Total Amino acids (mg/g)	Total Lipids (mg/g)	Total Phenols (mg/g)	OD Phenols (mg/g)
Red Stemmed Castor	180.09±20.40	23.12±4.56	24.80±7.53	0.35±0.01	5.12±0.12	9.29±1.80
Green stemmed castor	90.84±28.12	15.88±6.88	11.37±4.09	0.22±0.09	8.17±0.78	13.35±0.95

Table 2
Phenols and flavonoids identified in the leaves of two castor varieties

Varieties	Flavonoids	Phenols
Red stemmed castor	Kavaflavone, Apigenin, Kaempferol, Quercetin, Isovitexin.	Salicylic acid
Green stemmed castor	Apigenin, Isohaemetin, Kavaflavone, Zuteolin, Kaempferol, Isovitexin	Salicylic acid

Table 3
Biochemical profiles of haemolymph of *Pericallia ricini* reared on green stemmed castor leaves

Developmental Stages	Total Proteins (mg/g)	Total Carbohydrates (mg/g)	Total Amino acids (mg/g)	Total Lipids (mg/g)	Total phenols (mg/g)
III instar	3.10	21.0	0.03	0.32	1.20
IV instar	5.15	30.0	0.55	0.56	2.40
V instar	4.70	28.5	0.24	0.66	1.30

Table 4
Biochemical profiles of haemolymph of *Pericallia ricini* reared on red stemmed castor leaves

Developmental Stages	Total Proteins (mg/g)	Total Carbohydrates (mg/g)	Total Amino acids (mg/g)	Total Lipids (mg/g)	Total phenols (mg/g)
III instar	4.00	30.5	1.31	0.43	1.20
IV instar	6.55	31.5	2.20	0.62	1.60
V instar	5.60	37.5	1.44	0.71	2.00

Table 5
Total haemocyte count/mm^3 of *Pericallia ricini* reared on red and green stemmed castor leaves

Host leaves	III instar (No./mm^3)	IV instar (No./mm^3)	V instar (No./mm^3)
Red stemmed castor	8271± 261.1	6225±179.80	11856±282.8
Green stemmed castor	10500±282.42	8250±435.72	14714±271.5

as described above, from 1-3 individuals of each larval instar was drawn into Thoma White blood cell micro pipette upto a mark of 0.5 pricking the insects with the needle. It was mixed thoroughly with 20 times the volume of the Versene saline (NaCl-0.9 gm; KCl-0.942 gm; $CaCl_2$ -0.082 gm; $NaHCO_3$ -0.002 gm; Versene (EDTA)-2 gm; Dist. H_2O-100 ml) and introduced into the haemocytometer for five minutes, observations were made under the phase contrast microscope to estimate the total number of haemocytes present. For differential count of haemocytes, a smear was prepared on a clean glass slide, stained with Giemsa for five minutes, after that destained in tap water and observed under the microscope.

Results

Primary metabolites were quantitatively higher in the leaves of red stemmed castor as compared to the green stemmed variety (Table 1). The increases were 98% for carbohydrates, 45% for proteins, 118% for total aminoacids and 59% for total lipids. With regard to the secondary metabolites, 59% increase in total phenols and 44% increase in OD-phenols were observed in green stemmed castor.

Leaves of green stemmed castor showed two additional flavonoids, Isohaemetin and Zuteolin besides Kavaflavone, Apigenin, kaempferol, Quercetin and Isovitexin detected in the foliage of both red and green stemmed varieties. Salicylic acid was the only phenol found in both castor varieties (Table 2).

High levels of total aminoacids, proteins, carbohydrates and lipids were recorded in the haemolymph of different larval instars of *P. ricini* reared on leaves of red stemmed castor plants (Table 3 and 4). Levels of free amino-acids and proteins rose in the haemolymph of these larvae from 3rd to 4th instar reaching a peak and declined in the subsequent instars. A similar trend in the protein was noticed in the haemolymph of instars reared on green stemmed castor leaves. Carbohydrates gradually increased with progression in the larval development irrespective of whether the caterpillars were reared on the leaves of red or green stemmed castor plants. However, a maximum of 37.5 mg/g was registered in the 5th instar larvae fed on red stemmed castor leaves. As for the total phenols, an increase in the haemolymph of the insects reared on the green stemmed castor leaves was evident, from 3rd to 5th instar attaining a maximum (2.4 mg/g) in the 4th instar of the caterpillar.

Table 5 provides information relating to the total count of haemocytes in the various instars of *P. ricini* reared on the leaves of red stemmed and green stemmed castor plants. The total number of haemocytes were found to be maximum (14,714/mm^3) in the 5th instar

caterpillars fed on green stemmed castor leaves. Results of the differential count of haemocytes (Fig. 1) revealed that the individuals reared on the leaves of green stemmed castor showed higher percentage of prohaemocytes in III and V instar compared to counterparts reared on leaves of red stemmed castor. Other haemocytes such as granulocytes, adipohaemocytes, coagulocytes and oenocytoids were maximum in all the three instars when reared on leaves of green stemmed castor. On the contrary, percentage of plasmatocytes and spherule cells were highest in individuals raised on the leaves of green stemmed castor. Among the three instars, prohaemocytes and granulocytes showed an increase in the third instar, the rest of the haemocytes were more abundant in the fifth instar regardless of varietal differences in the castor leaves serving as food during development.

Discussion

The nutrient quality of the host plant directly influences the biochemistry of haemolymph which functions as a medium for the transport of digested and synthesized nutrients (Wyatt, 1980). Biochemical analyses of the haemolymph of *P. ricini* larvae reared on the leaves of red stemmed castor also showed an increased quantity of primary metabolites, indicating a positive correlation between primary metabolites level of the larval haemolymph and that of the insect's diet as observed by Bhatt and Krishna (1984) in *Corcyra cephalonica*. Increased quantities of primary metabolites in the haemolymph of 4th instar and a consequent decrease in the later larval stages of the insect support the observations on the wax moth *Galleria mellonella* (Lenartowitx et al., 1977).

Increase in the total haemocyte count of individuals reared on green stemmed castor leaves may be due to higher proportions of prohaemocytes and granulocytes in the haemolymph (based on the differential count study) which may be produced during phagocytosis as suggested by Gupta (1985; 1986). The increase in the number of plasmatocytes in the haemolymph of green stem fed individuals may be attributed towards the immune reaction against the toxic chemical produced by the plants (Price and Ratcliffe, 1974). The chemical substance, particularly "ricin" of castor leaves was found to influence the haemocyte morphology (Liener, 1979). The present study also revealed a maximum amount of secondary plant metabolites in leaves of green stemmed castor leaves which was positively correlated with haemocyte diversity in the larvae reared on this foliage. More number of prohaemocytes in the third instars may be due to the release from the haemopoietic tissue during young stages (Pathak and Vandana, 1993). The increase in the number of other haemocytes in the fifth instar can be attributed to the transformation of prohaemocytes into the other haemocyte types to satisfy the insect's physiological need (Beaulaton and Monpeyssin, 1976).

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Role of the Ectoparasite, *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) in the Suppression of *Sitophilus oryzae* and *Rhyzopertha dominica*

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Abstract: Role of the external parasite, *Anisopteromalus calandrae* (Howard) in suppressing the host populations of two major pests of stored wheat and rice namely, *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) were studied during June to September, 1989. The ability of *A. calandrae* to reach the hosts in wheat grains at the different depths were determined. The total per cent reduction in *S. oryzae* weevil population cultures maintained in the plastic containers in the laboratory with different number of introduced female parasites in the period of 4 months varied from 36.4±8.1% to 58.2±12.0%. When the mated female parasites were released at 1,5,10,20,40 and 50 introduction levels to each of 400 individual hosts, the parasites considerably restricted the growth of both the populations of *S. oryzae* and *R. dominica*. The introduction level of 20 mated female parasites resulted in 53% control of *S. oryzae* and nearly 45% control of *R. dominica* population was achieved with 5 female parasites. More progeny per parasite female were produced at the lower levels of introduction than at higher ones. Biological control of these 2 pests of stored wheat by *A. calandrae* may thus be considered as satisfactory.

INTRODUCTION

Control of insect pests by parasites, predators, pathogens etc. constitutes one of the essential elements of biological control (Hill, 1983). Success of entomophagous insects usually depends not only on the availability of their insect hosts but sometimes on a complex system of other factors such as additional food sources, alternate hosts and shelter (Greathead 1976). According to DeBach (1974), the most favourable combinations of natural enemies would be those that tend to parasitize or prey on different stages of the host. Mohyuddin and Shah (1977) reported that *Apanteles ruficrus* (Hym.: Braconidae) proved extremely efficacious against the noxious caterpillar, *Mythimna separata* (Lepidoptera: Noctuidae) in Pakistan and already saved thousands of pounds through protection of the pest attack.

The purpose of this study was to evaluate the suitability of the parasite, *A. calandrae* for use against *S. oryzae* and *R. dominica*, two notorious pests of stored wheat in tropical and subtropical climates.

MATERIALS AND METHODS

S. oryzae adults, larvae and pupae were collected from laboratory cultures maintained on wheat kernels at a constant temperature of 27±1°C which is considered favourable for its mass rearing (Sharifi and Mills 1971). *R. dominica* adults, larvae and pupae were mass cultured also on wheat kernels at a constant temperature of 30±1°C in an incubator for different experimental uses. Both the insects were mass-cultured in rectangular plastic containers (15 cm × 8 cm × 25 cm) on wheat grain having a moisture content of 12-14%. *A. calandrae* were stock cultured in the laboratory both on *S. oryzae* and *R. dominica* hosts for respective uses in the experiments on mass release of the parasites for suppression of the host populations.

To assess the capability of *A. calandrae* to suppress the population of *S. oryzae* and *R. dominica*, a series of experiments were con-

ducted.

a) To determine the ability of *A. calandrae* to reach the hosts in wheat grains, a rectangular plastic container 25 cm in height and 12 cm in diameter was entirely filled with fresh wheat kernels of which a depth of 5 cm from the bottom was filled with infested kernels of *S. oryzae* and *R. dominica* and newly emerged 20 males and 75 females were released at the surface. Then the container was covered with fine meshed cloth and tightly fastened with rubber band for proper ventilation and preventing parasites' easy escape. A small piece of cotton dipped in 50% honey solution inverted over the top for nourishment of the adult parasites was provided. The penetration time of the parasite to reach the bottom of the plastic container was carefully noticed. Then the depth of 5, 10, 15, 20 and 25 cm were checked for dead and live parasites and recorded. The experiment was repeated 3 times.

b) In another series of experiments, the columns of wheat grains of 100 cm depth and 7 cm diameter were prepared. Each column was marked at depths of 20, 40, 60, 80 and 100 cm. The bottom of the column was filled with a 5 cm-deep layer of grains containing *S. oryzae* and *R. dominica* infested kernels. Then 25 males and 70 females were released in each column at the top. The column was checked daily at different depths to record the presence of the parasites and the time to reach there. On the 5th day, the number of parasites that successfully reached the bottom layer were noted and whether eggs were laid in the host-containing grains or not, the grains of the bottom layer collected and incubated at 30°C for emergence of parasites, if any. The presence of emerged parasites in the infested grain was used as a measure of successful penetration of the parasite. This experiment was replicated 3 times.

c) In another experiment, a 9 feet tall column was made by attaching three hollow paper columns each of 3 feet high. A 10-cm layer of grain at the bottom were mixed with host-containing seeds. The column was marked at 1 ft interval by loosely placing papers. this facilitated the recording of the alive and dead parasites at different depths. The top and bottom was covered with fine meshed cloth for aeration after releasing newly emerged 50 male and 50 female parasites. On the 6th day, the column of wheat kernels was examined at the depth of each foot for confirming the ability of the parasite in successful penetration at different depths.

To study the efficiency of *A. calandrae* in checking *S. oryzae* population, two sets of weevil cultures each of which consisted of 7 jars were raised in the laboratory containing 100 gm of wheat. Fifty adult female weevils were introduced into 2 sets. One month after starting of the cultures 1,2,4,6,8 and 10 mated female parasites were released in 6 cultures and the 7th kept as a control. After nearly a month, samples of 200 grains were taken at 15 days interval from the middle of the culture with a glass tube about 1 inch in diameter. The adult weevils and parasites obtained in these samples were returned to the culture. Then the samples were incubated in the laboratory and the parasites that emerged during the 15 days and the weevils in one month's time were carefully noted and observations were continued for a period of 4 months.

To detect the suitability of *A. calandrae* for suppression of *S. oryzae*, 400 preferred hosts containing wheat kernels were kept; in petri dishes and thoroughly mixed with 200 gm of uninfested wheat grains. These samples, containing seeds of *S. oryzae* were then kept in an isolated sterilized and closed room (15 feet×10 ft) free from other natural enemies at 5 different places and a mated female was released in each sample and after 7 days interval, the parasites. If still remained, were removed from each sample and the grains of each sample were incubated in the laboratory for adult parasite emergence. The sexes and total number of parasite progeny thus produced from each sample were carefully recorded. Similarly, in other experiments having introduction levels of 5, 10, 20, 40 and 50 mated female parasites and with a fixed number of 400 seeds were conducted in the same manner successively. A sample containing 400 host seeds were kept in

the laboratory as a control for each experiment and the number of adult weevils emerged from the samples were counted and recorded.

To assess the suitability of *A. calandrae* for suppression of the population of the lesser grain borer, *R. dominica*, experiments were carried out with the introduction levels of 1, 5, 10, 20, 40 and 50 mated female parasites having 400 preferred host seeds in each sample. Each experiment was performed in the above mentioned procedure as for *S. oryzae* population control.

All the experiments were conducted during June to September, 1989 when the room temperature ranged from 28-30±1°C and 75±5% R. H. The data for the means were subjected to ANOVA and DNMRT.

RESULTS

From the experiment it is evident that when male and female adults were liberated at the surface of 25 cm deep layer of wheat kernels in a transparent plastic container, the females after 2-3 minutes of release, walked over the kernels and tried to locate host-seeds among grains. Due to the presence of a 20 cm layer of uninfested wheat grains from the top, it was quite difficult for the parasite to travel down. A careful and persistent observation revealed that 1 female reached the bottom of the container within 2.5 hours. Subsequently, it was found that out of 20 males, 3 males died at 10 cm depth, only 2 males reached the bottom successfully. Among the 75 females released at the top of the grain surface, 4 females died at 10 cm and 11 died at 15 cm depth but 30 females penetrated through the grains and reached the bottom of the column. The infested wheat kernels then after incubation produced 65 progeny showing successful oviposition of 30 females at the depth of 25 cm (Table 1). It was observed in another series of experiments performed for determining penetration capability of the parasite at a column depth of 100 cm that a maximum of 5 males died at the surface and 6 females died at a depth of 20 cm when newly emerged 25 males and 70 females were released at the top of the grain column.

Table 1. Ability of *A. calandrae* to reach the host containing wheat kernels at different depths in grain columns

Column depth tested	Depth of parasite penetration at various depths	Total no. of parasites released		No. of parasites detected				Time required for penetration at the bottom	Total no.of progeny produced from parasitized grains
				Alive		Dead			
		♂	♀	♂	♀	♂	♀		
25 cm	Top surface	20	75	1	1	-	--		
	5 cm			1	--	3	4		65
	10 cm			1	2	1	--		
	15 cm			3	2	--	11		
	20 cm			3	20	4	3		
	25 cm			2	30	1	2	2.5 hours	
Total				11	55	9	20		
100 cm	Top surface	20	70	3	7	5	4		
	20 cm			--	10	4	6		30
	40 cm			1	11	2	3		
	60 cm			1	12	4	5		
	80 cm			3	3	2	2		
	100 cm			--	5	--	2	57 hours	
Total				8	48	17	22		

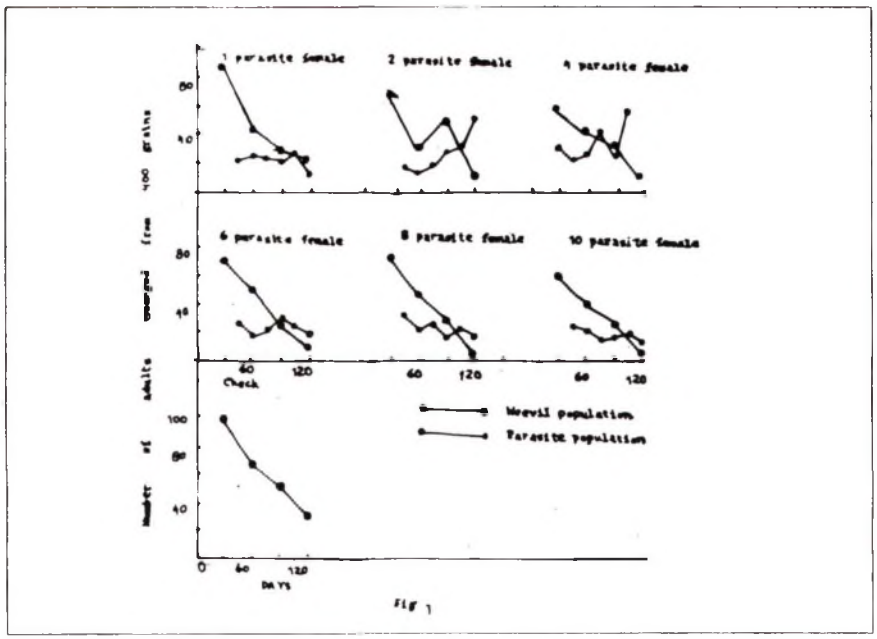

Fig. 1. Parasite and weevil population in cultures starting with 50 original mated adult female *S. oryzae*.

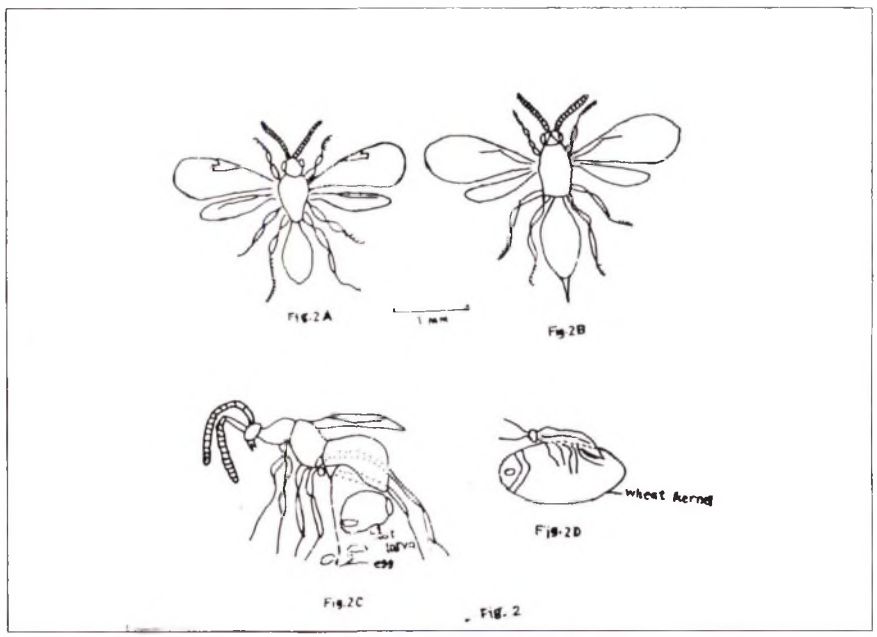

Fig. 2: 2A - An adult male parasite, *A. calandrae*; 2B - An adult female *A. calandrae*; 2C - Egg-laying of *A. calandrae*; on exposed *S. oryzae* host; 2D - Egglaying of *A. calandrae*; on hidden (inside wheat kernel) *S. oryzae* host.

Table 2. Mean per cent suppression of rice weevil, *S. oryzae* by mated *A. calandrae*

No. of parasite introduction	Total parasite progeny produced x ± S.E.	%		% suppression x ± S.E.	Progeny produced/female parasite x ± S.E.
		♂	♀		
1	86.8d* ± 4.7	41.3	58.6	21.7 ± 1.18	86.8 ± 4.72
5	185.6b ± 8.1	32.0	68.0	46.4 ± 2.04	37.1 ± 1.63
10	122.6d ± 10.3	52.6	47.4	30.6 ± 2.58	12.2 ± 1.03
20	212.2a ± 2.9	43.5	56.5	53.0 ± 0.72	10.6 ± 0.14
40	155.4c ± 7.5	59.1	40.8	38.8 ± 1.98	3.8 ± 0.18
50	131.2c ± 6.6	45.5	54.4	32.8 ± 1.68	2.6 ± 0.13

* In column, the means followed by the same letter are not significantly different (Ducan's New Multiple Range Test; P<0.01).

Males could not reach the bottom of the column but 5 females penetrated successfully and reached a bottom depth of 100 cm (Table 1).

It was recorded from the experiment that the female parasites reached upto a depth of 7 feet when tested at a column depth of 9 feet for penetration.

During the course of study on the efficiency of *A. calandrae* in controlling *S. oryzae* populations maintained in the laboratory culture, it was observed that both the host and parasite populations were low at the beginning of the experiment. The weevil population increased rapidly in comparison with the parasite population. Observations were continued for 4 months, and during this time, the grains were almost entirely destroyed by the weevils. The parasite population exceeded that of the host during the 4th month, perhaps because of shortage of food for the host. The number of adult parasite and host population is shown in Fig. 1. The total per cent reduction in weevil population with different number of introduced females in the period of 4 months varied from 36.4±8.10% to 58.2±12.0%. The maximum per cent reduction occurred in cultures starting with 8 parasite females.

Experimental studies to evaluate the effectiveness of *A. calandrae* for suppression of *S. oryzae* population in the sterilized room revealed that per cent suppression was highest at the introduction level of 20 parasite females, i.e., 53.0±0.72% (Table 2). The next average per cent suppression was 46.4±2.04% when 5 female parasites, the average per cent suppression of host population was lower, i.e., 32.8±1.68%. It is apparent from the results obtained that introduction levels with 5-50 female parasites always exhibited greater suppression than with introduction of single parasite female. It is also evident that more parasite progeny per female were produced at the lower introduction level than at the higher ones (Table 2). Maximal number of parasite progeny per female was produced with a single female, i.e.,86.8±4.72 and minimal with 50 females, 2.6±0.13. It is obvious that production of parasite progeny per female gradually decreases with the increase of the number of parasites released. Among the progeny produced, the number of female progeny was usually higher at different introduction levels and a maximum number of 68% was produced when 5 female parasites were introduced (Table 2).

In studies on the suitability of *A. calandrae* in the per cent reduction of *R. dominica* population, it is evident that maximum suppression of the host population was attained with the introduction level of 5 parasite females, i.e., 45.6±4.60 (Table 3). The next similar per cent suppression was observed when 20, 40 and 50 female parasites were released, i.e., 38.3±2.0, 35.5±2.1 and 34.3±2.2 parasite progeny produced respectively. Progeny production per female was always higher at lower levels of

introduction (Table 3). It is apparent that highest progeny per female was produced when a single female was introduced, i.e., 82.8±10.0 and the lowest progeny per female was found with the introduction level of 50 females, i.e., 2.7 ± 0.17. It is also evident that as with *S. oryzae* host, a similar trend of progeny per female occurred showing gradual decrease with the increase of parasite introduction levels. The introduction level of 20 mated female parasites resulted in 53% control of *S. oryzae* population. The highest male progeny was produced when a single female parasite released, i.e., 72.3% male and 27.6% female and the highest female progeny was produced with the introduction level of 50 parasite females, i.e., 53.5% female and 46.4% male.

Generally, *A. calandrae* females oviposited on *S. oryzae* and *R. dominica* hosts hidden inside wheat kernels. the parasite also laid eggs in scanty numbers on exposed 3rd and 4th instar larvae, prepupae and early pupae of *S. oryzae* host but oviposition did not occur in exposed host of *R. dominica*. The line drawing of adult male and female parasite, *A. calandrae* is given in Fig. 2A and 2B respectively. Egglaying on exposed and hidden host of *S. oryzae* (Inside wheat kernel) is shown in Fig. 2C and 2D respectively.

Discussion

From the present experiments it is evident that a female parasite always keeps herself busy in finding suitable hosts for parasitization even when the host were kept at 25 cm and 100 cm depth.

Ghani and Sweetman (1955) reported that *A. calandrae* females reached the host at a depth of 6 feet but failed to do so at 7 feet. in the present study, it was found that *A. calandrae* females could penetrate through a depth of 7 feet in search of host when tested on a column depth of 9 feet deep. Ghani and Sweetman (1955) also observed that males along with many females usually died at the top layers of the grain columns and no males were recovered below the first foot in wheat and 3.5 feet in corn. Their observation however, did not agree with the present findings, because 3 males reached upto a depth of 80 cm (Table 1). According to Ghani and Sweetman (1955), the differences in the depth reached in search of hosts through columns of wheat and of corn were evidently to differences in size and shape, as compared to wheat kernels, probably had larger interspaces and thus enables the parasite to crawl through them to greater depths in search of hosts.

During observations, it is found that *A. calandrae* is a strong flier and capable of dispersal for a considerable distance and has a wide range of hosts. Since the grain is stored in large quantities of 1000 kg or more by private and governmental agencies, the depth to which the parasite will disperse and penetrate in search of host is very important in evaluating its efficiency as a successful biological control agent. During a survey of the seasonal fluctuation of *A. calandrae* from the month of June to August, 1989, the parasite was recovered even from the bottom of gunny bags containing 100 kg wheat grains in ration shops and storages or shopkeepers with infested *S. oryzae* and *R. dominica*.

From the experiments of control efficiency studies in the laboratory of *A. calandrae*, against the rice weevil population, it is apparent that the maximum reduction of 58.2% host population was achieved with the introduction of 8 female parasites in 400 gm of wheat for a period of 4 months starting with 50 *S. oryzae* females. According to Ghani and Sweetman (1955), the total reduction in rice weevil population with the introduction of 1, 2, 3, 4, 5 and 6 pairs of *A. calandrae* females in 4 month's time varied from 34 to 59% starting with 40 original weevils and maximum reduction occurred in cultures starting with 5 pairs of female parasites. It was found that the *S. oryzae* population increased rapidly while the parasite population generally fell behind by 15 to 20 days.

Table 3. Mean per cent suppression of lesser grain borer, *R.dominica* by *A. calandrae*

No. of parasite introduction level	Total parasite progeny produced x ± S.E.	%		% suppression x ± S.E.	Progeny produced/female parasite x ± S.E.
		♂	♀		
1	82.8 ± 10.0c*	72.3	27.6	20.6 ± 2.50	82.8 ± 10.0
5	182.6 ± 18.5a	50.8	49.1	45.6 ± 4.60	36.5 ± 3.7
10	122.4 ± 5.4c	47.4	52.2	30.5 ± 1.35	12.2 ± 0.54
20	153.6 ± 8.3b	54.0	45.9	38.3 ± 2.09	7.6 ± 0.41
40	142.8 ± 8.6b	49.7	50.2	35.6 ± 2.17	3.5 ± 0.21
50	137.4 ± 8.6b	46.4	53.5	34.3 ± 2.24	2.7 ± 0.37

* In column, the means followed by the same letter are not significantly different (Ducan's New Multiple Range Test; P<0.01).

This slow rate of parasite reduction may be associated with the inability of the parasite to reach or locate hosts. The number of parasites was found to exceed that of host during the 4th month, perhaps because of shortage of good for the host insects and at the same time easy availability of the host larvae.

The parasite failed to control *S. oryzae* population in the laboratory culture effectively but delayed the destruction of the grain by the pest. This was probably due to its inability to reach or locate the majority of the hosts in the grain. According to Livingstone and Reed (1936) and Back (1939), the host larvae and pupae of *Lasioderma serricorne* (F.) that escape attack by the parasite, *A. calandrae* were able to reproduce and cause damage and thus contributed towards the rapid destruction of the grain.

During the course of studies on the suitability of A. calandrae for suppression of *S. oryzae* *R. dominica* populations, it was noted that an introduction level of 20 female parasites resulted in 53.0% control of *S. oryzae* hosts, whereas 45.6% control in *R. dominica* hosts was recorded when only 5 female parasites were released: However, here dead hosts were not counted. Hassel *et al.*, (1985) in their report included the dead hosts, *Callosobruchus chinensis* (L.) which had all been paralysed by *A. calandrae* and *Heterospillus prosopidis* Vier (Hym.: Braconidae) without successful oviposition. The desired control was not achieved because of the occurrence of superparasitism in *A. calandrae*. Often as many as 5 eggs were laid on a single host species but a single parasite emerged and the rest of the parasite larvae died out in competition. Here the female perhaps failed to discriminate properly the egg-containing hosts, previously laid by other competing females. In a similar study Press *et al.*, (1984) observed that *A. calandrae* suppressed rice weevil population by >90% established in wheat grain debris with the introduction level of 30-50 pairs of parasite and concluded that more progeny per parasite female were produced at the lower level of introduction. In the present study, a maximum 53.0% reduction was obtained. Production of more progeny per parasite female was observed at the lower levels of introduction than that of higher ones. This finding is in agreement with the results of Press *et al.*, (1984). Ghani and Sweetman (1955) however, did not get effective results when experimented with the weevils *S. oryzae* and *S. granarius* (L.) conducted in quart kerr jars in the laboratory. Dhaliwal (1975) reported that *a. calandrae* was able to limit host populations of *S. oryzae* quite effectively over a period of 4 months.

From out observations it is evident that *A. calandrae* suppressed about 45.6% *R. dominica* population (Table 3) with the introduction of 5 female parasites but at the introduction 10.50 females, the per cent suppression was comparatively less than the introduction of 5 females.

At higher introduction levels, much of the parasites' eggs are wasted through superparasitism and intraspecific competition. The per cent suppression of *R. dominica* was lower than that of *S. oryzae* population. Chatterji (1955) also reported that of the two cereals pests, namely *S. oryzae* and *R. dominica*, the former can be better controlled by *A. calandrae* restricting the growth of *R. dominica* population in the laboratory culture when these already grew to a damaging level.

Press (1989) found that anthocorid predator, *Xylocoris flavipes* Reuter and ichneumonid parasite, *Venturia canescens* to be compatible for use as biological control agents against stored product pyralids such as *Cadra cautella* (Walker). Mohyuddin and Shah (1977) described that *Apanteles ruficrus* (Hym.: Braconidae) provides an example of a geographical bio-type of a natural enemy giving the necessary control. *A. ruficrus* was introduced from Pakistan and 40,000 adults were released in 1971 and 1972 in New Zealand against the noxious caterpillars *Mythimna separata* and *Agrotis* spp. (Lepidoptera: Noctuidae). The parasite found to be highly effective and saved a lot of money through reduction of pest incidence. Banks and sharp (1979) reported that the pteromalid wasp, *A. calandrae* played an important role in controlling *Rhizopertha dominica*.

Analysing the performance of *A. calandrae* in checking its host populations, it can be concluded that although the parasite can markedly suppress its host, a satisfactory control can not always be expected. It is likely that to achieve desired results a much more vigorous approach is required based on adequate biological and ecological studies which are at present are largely lacking.

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ENTOMON 1995 VOL. 20 NO. 3 & 4: 183-189

Digestive Enzymes and Regional Localisation of Proteolytic Endopeptidases in the Alimentary canal of the Kola Nut Weevil, *Sophrorhinus insperatus* Faust (Coleoptera: Curculionidae).

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Abstract: The digestive enzymes present in the alimentary canal and the salivary glands of *Sophrorhinus insperatus* Faust were surveyed using qualitative detection methods. A wide range of digestive enzymes were detected in the gut while fewer enzymes were found in the salivary glands. Alpha and beta-glucosidase, alpha-galactosidases, amylase, trypsin, lipase and weak cellulase activity were detected in the gut homogenate of the weevil. The salivary glands of the kola weevil contained only alpha glucosidase, alpha-galactosidase, amylase and trypsin. The use of synthetic substrates revealed the presence of trypsin-like and chymotrypsin-like enzymes and electrophoretic studies implicated the anterior and posterior midguts as the sites of occurrence of endopeptidases.

Keywords: *Sophrorhinus insperatus*, digestive enzymes, electrophoresis, endopeptidase, amylase, glucosidase, lipase, cellulase.

INTRODUCTION

Sophrorhinus insperatus Faust is a principal pest of kola throughout the kola-producing areas of West and Central Africa (Albert and Mallamaire, 1955; Daramola, 1974). Weevil infestation is usually initiated in the field and it is carried into a storage where it continues its destructive activities. The feeding and developmental activities of *Sophrorhinus* render the kola nuts unfit for human consumption and industrial use. Since little success has been achieved in the field control of the weevils (Gerard, 1967; Ivbijaro, 1978), measures aimed at controlling them have, been restricted to the stores.

The importance and relevance of digestive physiology to the control of insects have long been recognised (Uvarov, 1966; Ishaaya and Swirski, 1970). In spite of the ample amount of information available on the digestive enzymes in insects (House, 1974; Applebaum, 1985), there is a dearth of information on the physiology of *S. insperatus* in general and its digestive physiology in particular. It is against this background that investigations were carried out to survey qualitatively the digestive enzymes that are present in the guts of the larva and adult as well as the salivary glands of the adult *S. insperatus* with the hope of providing basic data for the analysis of damage and possible control strategies.

MATERIALS AND METHODS

Insect Cultures: Initial cultures were obtained from field infested kola nuts whose weevils were reared to the adult stage in baskets lined with banana leaves. Subsequent cultures were raised from the original stock by caging clean kola nuts with at least twenty tenurial adults of both sexes in rectangular plastic cages measuring $13.5 \times 8.5 \times 6.2$ cm. The plastic cages were constructed as described by Daramola (1978). Each plastic cage has a removable top on which there are two holes of 3cm diameter covered with muslin cloth. On each side of the cage was a 3cm diameter hole, three of which were covered with muslin cloth to allow for adequate aeration. Water was provided for the insects through the fourth hole. The kola nuts from the plastic cages were pooled after two weeks and kept in baskets lined with banana

leaves. The last larval instar and 7-day old adults were used throughout the experiment.

Preparation of homogenate: The larvae or adults of *S. insperatus* were asphyxiated in a deep freezer for thirty minutes and their guts were carefully dissected out. Whole guts of either stage of *S. insperatus* were homogenized in 0.5ml distilled water in an all-glass homogenizer. The homogenate was adjusted to 4 guts/ml with distilled water and the homogenate was centrifuged at 4000 rev/min for five minutes. The resulting supernatant was used for enzyme assays. Salivary gland homogenates were similarly prepared and adjusted to 16 pairs of salivary glands/ml.

Determination of enzyme activities: The method described by Balogun (1972) was employed for the determination of carbohydrase activities. Carbohydrates were assayed in a reaction mixture consisting of 1.0ml of 4% phosphate buffered substrate (pH 6.5) and 1.0ml of enzyme extract. The substrates used were starch, raffinose, sucrose, cellobiose, trehalose, lactose, maltose, salicin, carboxymethyl cellulose (CMC), filter paper, chitin and inulin. Two drops of toluene were added to the reaction mixture to prevent bacterial activity and all experiments were duplicated. In the control, the enzyme solution was heat inactivated for 20 minutes in a boiling water bath prior to its addition to the reaction mixture. The tubes containing the reaction mixtures were incubated for 6-12 hours at 37°C. The products of hydrolysis were determined chromatographically. A quantity (40 ul) of the reaction mixture and marker sugars were applied at 20cm apart on Whatman No. 1 chromatographic paper and the products of hydrolysis resolved in ascending manner using n-butanol-acetic acid-water (4:1:5V/V) as solvent. The air-dried chromatograms were dipped in aniline phthalate solution prepared according to Patridge (1949). The chromatograms were air-dried and heated for 10 minutes at 105°C in an oven to detect the reducing sugar spots.

The hydrolysis of the polysaccharides and non-reducing dissaccharides was determined in terms of the appearance of reducing properties using the Benedict's test. An aliquot (5.0ml) of the alkaline copper reagent of Benedict was added to (1.0ml of the reaction mixture and heated for 20 minutes at 100°C in a waterbath. The appearance of a brick-red precipitate was taken as an index of a positive reaction.

Digestive proteinases and lipases present in the gut enzyme extract of *S. insperatus* were determined qualitatively according to the method described by Balogun and Fisher (1970). Trypsin like activity was estimated in a reaction mixture containing 0.5ml alkaline casein (pH 7.6) and 0.5ml of the enzyme solution while acidic casein (pH 2.0) was employed for the determination of pepsin-like activity. Incubation was for 6 hours and 1% acetic acid was added drop by drop to both test and the control. Increase in turbidity of the test solution was taken as an index of tryptic activity. The test solution was replaced with 10% sodium acetate in case of pepsin. Lipolytic activity was estimated in a reaction mixture containing equal volumes (1.0ml) of 25% olive oil emulsion (pH 7.0) and enzyme solution. Denatured enzyme solution was used to replace the enzyme extract in the control tube. After 12 hours incubation at 37°C, 3.0ml of 95% ethanol and two drops of phenophthalein were added to the test and the control tubes. The reaction mixtures were titrated against 0.05N NaOH to a similar pink colour. Increase in the titre value of the test mixture compared with the control was taken as an index of lipase activity.

Electrophoresis: The alimentary canal was divided into 4 regions namely, foregut, anterior and posterior midguts and hindgut for the purpose of regional localization of proteins and proteolytic enzymes. Electrophoretic analysis of the different gut regions were carried out in precibor tubes (7.5 × 0.7cm bore) containing 12.5% polyacrylamide gel prepared in accordance with the procedures described by Webber and Osborn (1975). An aliquot of the sample was applied to the gel and electropho-

retic movement was from cathode to anode at a constant current of 2mA per gel. The gels were stained with Coomassie Brilliant Blue R250 for 2h and destained in methanol: acetic acid: water (50: 75: 87, 5 V/V/V). The resulting protein bands were characterised into trypsin-like and chymotrypsin-like enzymes based on the hydrolysis of synthetic substrates. The synthetic substrates tested include p-tosyl-L-arginine methyl ester hydrochloride (TAME), Benzoyl-L-tyrosine ethyl-ester (BTEE) and N-Benzoyl-arginine-ethyl ester (BAEE). Digestion of TAME and BTEE were estimated by the methods of Hummel (1959) at 248nm and 258nm for TAME and BTEE respectively. The methods of Schwet and Takenaka (1955) were employed for the determination of activity towards BAEE at 253nm. Detailed descriptions of these procedures have been given elsewhere by Adedire (1994).

Results and Discussion

Results obtained from the survey of the digestive enzymes in the guts of the larva and adult kola weevil, *S. insperatus* (Table 1) indicated the presence of enzymes that are capable of hydrolysing sucrose, maltose, trehalose, raffinose, salicin, cellobiose, filter paper, CMC, alkaline casein and olive oil emulsion. The higher enzyme activities detected in the larval gut homogenate is expected since the larval stage is the most destructive stage of the weevil.

The wide range of carbohydrases detected in the guts of the weevil have correlations with the high carbohydrate content of kola nuts as reported by Ogutuga (1975). The wide variety of carbohydrates digested may indicate group specificities rather than the occurrence of the specific substrates in the normal diet. Similar observations have been made by Barrington (1962) and Morgan (1975). Some of the observed results can be rationalised. For instance, sucrose is found widely in the plant kingdom (Pigman and Horton, 1970) and its products of hydrolysis (glucose and fructose) are presumed to be vital nutritional value to insects. The high trehalase activities observed in both stages of the weevil probably depicts the vital role of trehalose as important metaic

Table 1. Hydrolysis of various food substances by homogenates of larval and adult guts and adult salivary glands of *Sophrorhinus insperatus*.

Enzyme	Substrate	Larva	Adult	Salivary gland
General α-glucosidase	Sucrose	+	+	++
	Maltose	+	+	+
Specific α-glucosidase				
α-α-trehalase	Trehalose	+	+	+
β-glucosidase	Cellobiose	+	+	-
	Salicin	+	+	-
α-galactosidase	Raffinose	++	+	+
β-galactosidase	Lactose	-	+	-
Amylase	Starch	++	-	+
Cellulase	Carboxymethyl		+	-
	Cellulose	+	+	
	Filter Paper	+	-	-
Chittinase	Chitin	-	-	-
Inulinase	Inulin	-		-
Trypsin	Alkaline Casein	+	+	+
Pepsin	Acid casein	-	-	+
Lipase	Olive oil emulsion	+	+	-

(++) Strongly positive reaction; (+) Positive reaction; (-) No reaction

bolic fuel (Wyatt, 1967). The high alpha galactosidase activity detected is expected since raffinose occurs widely in the plant kingdom but in lower amount than sucrose (Pigman and Horton, 1970). The absence of lactose-hydrolysing enzyme could be due to the absence of substrate in the normal diet of the insect since lactose is rare in higher plants (Pigman and Horton, 1970). High amylolytic activity detected in the guts of the weevil is expected since starch constitutes about 33% of the dry matter content of kola nuts (Beattie, 1970). The end-products of starch hydrolysis (maltose and isomaltose) can be digested by alpha-glucosidases. This also holds for cellulose whose end-products (cellobiose) can be hydrolysed by beta-glucosidase (cellobiose). Although cellulase activity is a pre-requisite for cellobiose activity, cellulolytic activity is extremely weak in *S. insperatus*. However, carboxymethyl-cellulase activity is somewhat higher probably CMC is more easily suceptible to hydrolysis. Since the guts of the weevils were not screened for microbial activity, the weak cellulase activity observed could either be of animal or microbial origin or both. However, cellulase activity may be of great nutritional value during starvation. The slighly higher carboxymethyl-cellulase activity in the adult is probably acquired in order to cope with the digestion of cellulose in the testa of kola nuts before the weevil can gain access to the nuts. The weak cellulase activity is probably responsible for the inability of some kola weevils to infest undamaged pods as observed by Daramola (1978). The absence of chitinase might be a protective measure to avoid autodigestion since the alimentary tract is lined with chitin.

These *in vitro* studies showed that the digestive apparatus of *S. insperatus* contained only alkaline proteinases. This result is consistent with the observations of several other workers (Balogun, 1969; House, 1974; Baker *et al.*, 1984a; Applebaum, 1985). The amount of lipase activity detected in the alimentary canal of the kola weevil is generally low probably due to low amount of extractable oils in kola nuts as recorded by Ogutuga (1975) and Ogutuga and Daramola (1977). Similarly, low lipolytic activity had been reported in the midgut of the larch bark beetle by Balogun (1969). Although it is debatable whether the enzymes detected in the alimentary tract of this weevil are secreted or not, it is fairly wellknown that intracellular digestion is absent or rare in insects (Barnard and Prosser. 1973).

Table 1 also showed the occurrence of alpha-glucosidase, amylase and trypsin in the salivary glands of the adult kola nut weevil. Although Dannel (1946) suggested that the salivary glands of *Sitophilus* (=*Calandra*) *granarius* (L) do not produce copious secretion, evidences acruing from starvation and subsequent feeding by Baker *et al.*, (1984b) indicated slow but continuous secretion of amylase by the salivary glands of *S. granarius*. It is evident from this study however, that the salivary glands in *S. insperatus* play an important role in the primary digestion of ingested kola nuts.

The electrophoretic variation of the protein bands in the various regions of the alimentary canal of adult weevil is shown in Figures 1a and 1b. The anterior midgut contains about ten protein bands. The bands in the various regions were characterised using synthetic substrates. Activities were detected mainly in the fast-moving anodal components. In the anterior and posterior midguts, the fastest moving band greatly hydrolysed TAME and BAEE. It also exhibited slight activity towards BTEE.

It was observed that the second fast-moving anodal band only hydrolysed BAEE and the activity was quite high. Chymotryptic activity was only detected in bands 3 and 4 of the anterior midgut following slight hydrolysis of BTEE. None of the bands detected in the fore- and hindguts hydrolysed any of the synthetic substrates.

Polyacrylamide gel electrophoresis of the various gut regions of the larva of *S. insperatus* showed variation in the protein patterns of each gut region (Figures 2a, b).

The trend of this result is similar to the one obtained for the adult weevil but for some slight qualitative and quantitative differences in

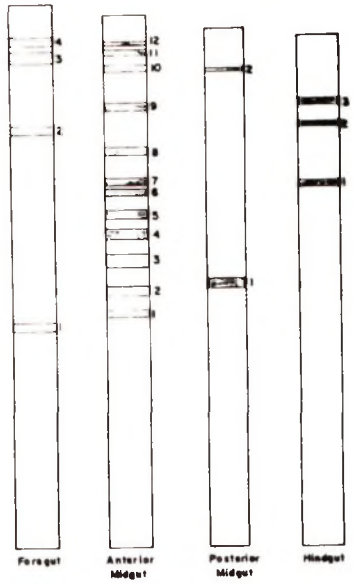

Figure 1b Diagramatic illustration of the electrophoretic patterns of the proteins in the various regions of the gut of the adult Sophrorhinus insperatus Faust

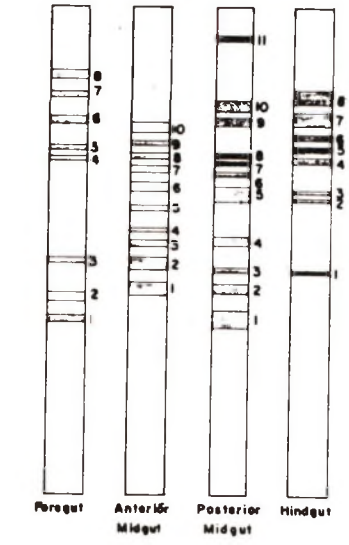

Figure 2b Diagramatic illustration of the electrophoretic patterns of the proteins in the various regions of the gut of the larva of Sophrorhinus insperatus Faust

Fig. 1a: Electrophoretic patterns of the proteins in the various regions of the gut of adult *S. insperatus* Fasust.; Fig. 1b: Diagrammatic illustration of the electrophoretic patterns of the proteins in the regions of the gut of adult *S. insperatus* Faust.; Fig. 2a: Electrophoretic patterns of the proteins in the various regions of the larva of adult *S. insperatus* Fasust.; Fig. 2b: Diagrammatic illustration of the electrophoretic patterns of the proteins in the regions of the larva of adult *S. insperatus* Faust.

the protein bands. It is pertinent to mention the occurrence of two very faint fast-moving anodal bands in the foregut of the larva of the weevil. These bands had similar relative mobilities as those found in the midgut regions. The bands hydrolysed TAME and BAEE. Their occurrence in the foregut may be due to backflow from the anterior midgut. The protein bands are generally more distinct in the larval extract than in the adult especially in the posterior midgut.

Bands 3 and 4 at the posterior midgut slightly hydrolysed BTEE. None of the protein bands detected in the hindgut preparation hydrolysed any of the substrates tested. The results of this study corroborates an earlier investigation by Adedire (1994) who showed that the anterior and posterior midguts were the main sites of carbohydrate and protein digestion with higher activity levels of digestive proteases in the posterior midgut. Adedire (1990) had also shown that the fastest moving anodal band contained trypsin like enzymes which also tallies with the observations made in this study.

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Bioecology of *Harmonia eucharis* (Mulsant) (Coleoptera: Coccinellidae). An Aphidophagous Predator in Western Himalayas

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Abstract: *Harmonia eucharis* when feeds on *Brachycaudus helichrysi, Eriosoma lanigerum* and *Macrosiphoniella pseudoartemisiae* lays 618±62.58, 576.14±65.45 and 549.71±66.04 eggs respectively during its 44-51 days of oviposition period. Its total duration of preimaginal development at 23.8±0.50°C are 21.43±1.92, 24.86±3.09 and 27.43±1.84 days and at 17.9±1.70°C are 34.29±1.83, 38.14±2.36 and 41.29±2.60 days; larvae at 23.7±0.30°C consume 668.86±47.83, 646.14±37.35 and 623.86±40.59 and at 17.3±1.60°C consume 800.14±45.05, 760.14±23.93 and 712.71±22.19 of the above mentioned prey species respectively. The calculated lower temperature limit for the entire development is 23.3°C and the sum of effective temperature is 338 days degree. *H. eucharis* has two generations in an year.

Key words: Aphidophagous beetles, Garhwal Himalaya, life stages, development, voracity, survival, logevity and fecundity.

INTRODUCTION

Harmonia eucharis (Mulsant), a coccinellid beetle is found feeding on different species of aphids in Western and Northwest Himalayas. Life stages, developmental rate, larval voracity and oviposition of this beetle are studied to assess the potentiality of this predator as bio-control agent of aphids.

MATERIALS AND METHODS

General life stages of *H. eucharis* were studied from the specimens obtained from rearing stock and also from random field surveys. The adults obtained from the hibernating pupae collected in April, 1984 at Joshimath (1875m), Western Himalaya were reared in the laboratory on three different aphid species viz. *Brachycaudus helichrysi* (Kaltenbach), *Eriosoma lanigerum* (Hausman) and *Mcrosiphoniella pseudoartemisiae* (Shinji) for different experiments.

Demographic analysis was started with freshly laid eggs (n=94). The newly hatched larvae (n=7) were reared separately and provided with sufficient number of fourth instar nymphs of specific aphid species as food in order to note the preimaginal and imaginal development along with the records on survival.

The lower limit of temperature threshold and total thermal requirement for each developmental stages were calculated using the same formula as adopted by Bodenheimer and Swirski (1957), Hodek (1973) and Chakrabarti *et al.*, (1988 and 1991).

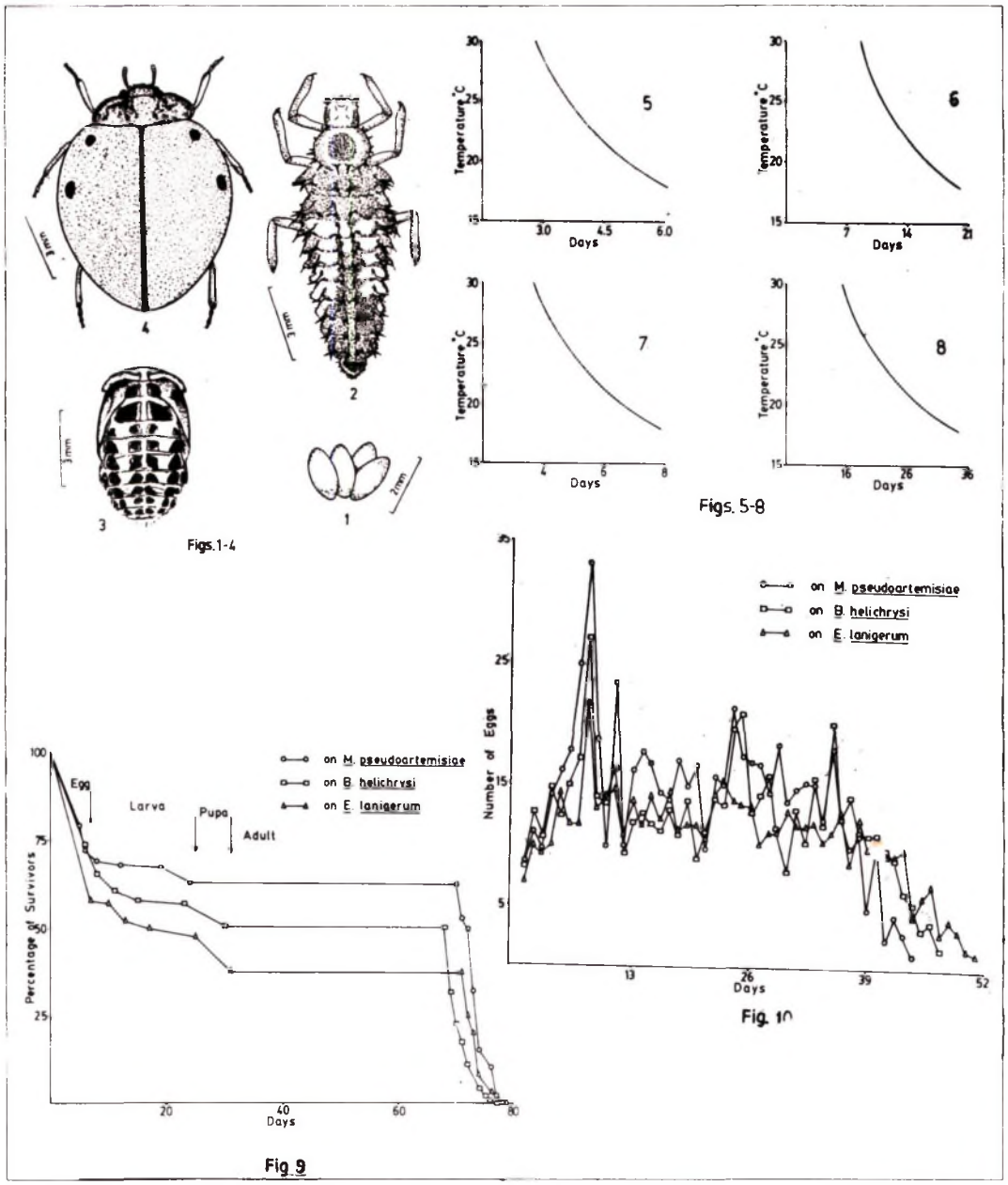
The rate of larval consumption was calculated at every 24 hours interval. Oviposition experiment started with the laying from first generation adults. Mated females were provided with fourth instar nymphs of specific aphid species and with fresh plant parts for ovipositon stimulus. Sexes of *H. eucharis* were not considered for the study on longevity.

RESULTS AND DISCUSSION

Life Stages:

This species completes its life cycle through 4 stages as mentioned below:

i. **Egg** (Fig. 1): The freshly laid eggs are elongated oval in shape, 1.48-1.88mm in length and 0.74-0.79mm in breadth and yellowish in colour. Eggs are laid in cluster.



Figs. 1-4: *Harmonia eucharis* (Mulsant): 1. Egg; 2. Fourth instar larva; 3. Pupa; 4. Adult.

Figs. 5-8: 5. Effect of temperature on preimaginal development of *Harmonia eucharis* (Mulsant) feeding on *Macrosiphoniella pseudoartemisia*. 5. Effect of temperature on development of eggs feeding on *M. pseudoartemisiae*; 6. Effect of temperature on development of larva feeding on *M. pseudoartemisiae*; 7. Effect of temperature on development of pupa feeding on *M. Pseudoartemisiae*; 8. Effect of temperature on different developmental stages feeding on *M. pseudoartemisiae*.

Fig. 9. Percentage of survival of *Harmonia eucharis* feeding on *Macrosiphoniella pseudoartemisiae, Brachycaudus helichrysi* and *Eriosoma lanigerum*.

Fig. 10. Average daily oviposition rate in Harmonia *eucharis* feeding on *Macrosiphoniella pseudoartemisiae, Brachycaudus helichrysi* and *Eriosoma lanigerum*.

Table 1. Duration in days (Mean ± SD) (n=7) of immature stages of *Harmonia eucharis* reared on different aphid species. at a) 23.8 ± 0.50°C and b) 17.9 ± 1.70°C (Ranges are in paranthesis)

Aphid species	Egg	Larval Instar				Total	Pupa	Total
		I	II	III	IV			
Macrosiphoniella pseudeartemisiae	a) 3.86 ± 0.83 (3 - 5)	1.43 ± 0.49 (1 - 2)	1.71 ± 0.45 (1 - 2)	3.86 ± 0.64 (3 - 5)	5.43 ± 0.75 (5 - 7)	12.43 ± 1.30 (10 - 14)	5.14 ± 0.64 (4 - 6)	21.43 ± 1.92 (18 - 24)
	b) 6.14 ± 0.64 (5 - 7)	3.43 ± 0.73 (3 - 5)	2.86 ± 0.64 (2 - 4)	5.14 ± 0.64 (4 - 6)	8.86 ± 0.83 (8 - 10)	20.29 ± 1.28 (19 - 23)	7.86 ± 0.83 (7 - 9)	34.29 ± 1.83 (32 - 38)
Brachycaudus helichrysi	a) 4.71 ± 1.03 (3 - 6)	2.29 ± 0.70 (1 - 3)	2.71 ± 0.70 (2 - 4)	4.29 ± 0.88 (3 - 6)	5.57 ± 1.18 (4 - 8)	14.86 ± 2.03 (12 - 18)	5.29 ± 1.03 (4 - 7)	24.86 ± 3.09 (20 - 30)
	b) 7.14 ± 0.99 (5 - 8)	4.43 ± 0.73 (4 - 6)	3.29 ± 0.45 (3 - 4)	5.57 ± 0.90 (4 - 7)	9.29 ± 0.88 (8 - 11)	22.57 ± 1.40 (21 - 25)	8.43 ± 0.90 (7 - 10)	38.14 ± 2.36 (33 - 41)
Eriesemn lanigerum	a) 5.29 ± 1.03 (4 - 7)	2.86 ± 0.64 (2 - 4)	3.29 ± 0.70 (3 - 5)	4.29 ± 0.45 (4 - 6)	5.86 ± 0.83 (5 - 7)	16.29 ± 1.39 (14 - 19)	5.86 ± 1.12 (5 - 8)	27.43 ± 1.84 (25 - 31)
	b) 7.86 ± 1.12 (7 -10)	5.14 ± 0.64 (4 - 6)	3.71 ± 0.70 (3 - 5)	6.00 ± 1.07 (5 - 8)	9.57 ± 1.18 (8 - 12)	24.43 ± 1.76 (22 - 27)	9.00 ± 1.20 (7 - 11)	41.29 ± 2.60 (38 - 45)

Table 2. Consumption (Mean ± SD) (n=7) of different prey aphid by the instars of *Harmonia eucharis* at a) 23.7 ± 0.30°C and b) 17.3 ± 1.60°C (Ranges are in paranthesis)

Aphid species	Larval Instar				Total	Average daily
	I	II	III	IV		
Macrosiphoniella pseudeartemisiae	a) 9.29 ± 1.83 (6 - 12)	37.14 ± 9.43 (20 - 54)	127.14 ± 17.92 (94 - 156)	495.43 ± 36.05 (452 - 558)	668.86 ± 47.83 (594 - 737)	53.82
	b) 25.86 ± 6.13 (17- 34)	58.29 ± 9.91 (44 -72)	157.86 ± 12.98 (140 - 185)	558.14 ± 19.98 (526 - 590)	800.14 ± 45.05 (727 - 381)	39.44
Brachycaudus helichrysi	a) 8.14 ± 2.10 (4 - 11)	33.71 ± 9.75 (17 - 48)	121.71 ± 17.88 (86 - 147)	482.57 ± 33.37 (442 - 543)	646.14 ± 37.35 (602 - 716)	43.49
	b) 22.43 ± 4.59 (18 - 32)	48.43 ± 8.47 (33 - 59)	150.00 ± 12.07 (129 - 166)	539.29 ± 16.19 (511 - 562)	760.14 ± 23.93 (724 - 789)	33.68
Eriesemn lanigerum	a) 6.71 ± 2.05 (3 - 9)	26.86 ± 7.32 (14 - 37)	116.71 ± 21.19 (78 - 139)	473.57 ± 31.99 (436 - 532)	623.86 ± 40.59 (572 - 704)	38.31
	b) 19.43 ± 4.14 (15 - 28)	36.57 ± 7.27 (26 - 49)	137.43 ± 9.44 (120 - 148)	519.29 ± 16.52 (495 - 547)	712.71 ± 22.19 (674 - 739)	29.18

ii.**Larva**: The larva passes through four (4) different stages.

First instar larva: It is elongated 2.15-2.50mm in length and 0.61-0.89mm as maximum width. Body is chitinised and brown in colour. Head is rounded, fused with prothorax; dorsocephalic hairs long and pointed. Antennae are three segmented. Thorax is rugose; pro and mesothrorax are fused together; metathorax is with paired medial and lateral senti. Legs are deep brown in colour; tibio-tarsal segment is elongated and with claw. Abdomen is 9-segmented, rugose and sclerotised. Each segment is with paired spinal and lateral parascoli; dorsal hairs are long and pointed.

Second-Third instar larva: The second instar larva is 5.40-6.00mm in length and 1.20-1.95mm in width. The third instar larva is 8.10-8.55mm in length and 1.80-2.85mm as maximum width. The larvae of these instars

are similar to that of the first instar larva almost in all respects except in size.

Fourth instar larva (Fig. 2): Body is 10.10-11.65mm in length, 2.56-3.25mm in width, deep brown in colour and highly sclerotised. Each abdominal segment is with one pair of spinal parascoli and two pairs of lateral parascoli. Each spinal and lateral parascoli is with 2-3 and 2 scoli respectively; dorsomedial hairs are small, thick and blunt below the parascoli; the lateral hairs are long, finely pointed are arising from elevated area. Ventral hairs are more numerous and longer than the dorsal hairs. Other characters are as in the first instar larva.

iii. **Pupa** (Fig. 3): Body is elongated oval, 8.26-8.99mm in length and 4.22-4.97mm in breadth, deep brown in colour with some segmental reddish areas. Head is with spinules on the anterior margin and smooth on the posterior margin; dorsum of head is with small hairs. Abdomen is membranous; dorsal abdominal hairs are small and pointed.

iv). **Adult** (Fig. 4): Mulsant (1853) described the adult morphology of this species.

DEVELOPMENT

Feeding on *M. pseudoartemisiae*, the period of preimaginal development (Table 1) was shorter at high temperature and also observed the shortest when compared with other two prey species. The lower threshold for development was 7.9°C for the eggs, 8.6°C for the larvae and 6.8°C for the pupae irrespective of the prey aphids. The hyperbola (Figs. 5-8) was obtained from the values of the thermal constant for egg (61 dd), larval (189 dd), pupal (88 dd) and total preimaginal (338 dd) development only for *M. pseudoartemisiae*. Similar development period has been reported in *Adalia bipunctata* L. (Clausen, 1915; Ellingsen, 1969), *Coccinella undecimpunctata* L. (Benham and Muggleton, 1970), *C. septempunctata* L. (Tao and Chiu, 1971), *L. dimidiata* (Fab.) (Tao and Chiu, 1971; Chakrabarthi *et al.*, 1988) and *Propylea 14-punctata* (L.) (Rogers *et al.*, 1972).

SURVIVAL

The survivorship graph (fig. 9) shows that the mean percentge of hatchability of egg in *H. eucharis* at 20.8±3.30°C is 73.67±4.50. This egg hatchability was higher than *Coccinella septempunctata brucki* Mulsant and *Propylea japonica* (Thunberg) (Kawauchi, 1985 and 1990), *Menochilus sexmaculata* (Saha, 1987), *Micrapis discolor* (Fab.) (Agarwala *et al.*, 1988) and lower than *Verania discolor* (Islam and Nasiruddin, 1977) but similar to *Propylea 14-punctata* (Roger *et al.*, 1972). The rate of survival of larva and pupa were 71-85% and 80-94% respectively on these different prey aphids studied. Maximum larval mortality occurred in the first instar (9-19%) but was low in the third instar (2-4%).

LONGEVITY

The adult longevity of *H. eucharis* (Fig. 9) was recorded as 47-61 days (fig. 9). It is similar to *Cycloneda limbifer* Casey (Zeleny, 1969) but higher than *M. sexmaculata* (Rajmohan and Jayaraj, 1974) and *C. septempunctata* L. (Singh and Malhotra, 1979). However, Saha (1987) recorded 108-112 days longevity of females and 80-84 days of males in *M. sexmaculata*.

OVIPOSITION

Observation was recorded when the first generation adults started ovipositing. It began about a week after the emergence of adults and was continued for 44-51 days having maximum on the 8th days. The oviposition rate was highly variable and ranged from 0-38 eggs/day (Fig. 10). Similar oviposition period has been recorded in *Scymnus nubilus* Mulsant (Johnson, 1973), *Scymnus interruptus* Mulsant (Tawfik *et al.*, 1973); *S. hoffmanni* Weise (Kawauchi, 1990). But the period were higher in *M. sexmaculata* (Saha, 1987), *C. septempunctata* and *Propylea japonica* (Kawauchi, 1990) and lower in *Adalia bipunctata* (Clausen, 1915; Ellingsen, 1969), *C. septempunctata* (Singh and Malhotra, 1979).

The average total fecundity differed be-

tween 549.7 and 618.0 with 465 as lowest and 716 as highest number during their life time. It is similar to *C. septempunctata* (Sunbdy, 1969; singh and Malhotra, 1979), *M. discolor* (Agarwala *et al.*, 1988) but lower in comparison to *Cycloneda limbifer* (Zeleny, 1969), *C. septempunctata and P. japonica* (Kawauchi, 1985) and higher than *A. bipunctata* (Aleksidze, 1970), *Scymnus nubilus* (Johnson, 1973), *S. interruptus* (Tawfik *et al.*, 1973), *S. hoffmanni* (Kawauchi, 1990).

The results indicated that *Harmonia eucharis* preferred *M. pseudoartemisiae* for egg laying since it produced highest number of eggs (Fig. 10) as compared to *B. helichrysi* and *E. lanigerum*.

LARVAL FOOD CONSUMPTION

Depending on the temperature and developmental stages (Table 2), the food consumption of the larvae of *H. eucharis* on three aphid species were different. At high temperature, the daily feeding rate was higher but total feeding rate was lower than at low temperature. The first instar larvae consumed 6.7-25.9 aphids (average number) i.e., 1.1-3.2% of aphids larval consumption while the fourth or the final instar larvae consumed maximum number of prey i.e., nearly 70% aphids. The total aphid consumption by a larva of this beetle was similar with *Coccinella 5-notata L.*(Palmer, 1994), *L. dimidiata* (Tao and Chiu, 1971; Chakrabarti, *et al.*, 1988), *C. septempunctata* (Agarwala and Saha, 1986).

Cannibalism was found in this species as shown by Bose and Ray (1967), Dimetry and Mansovr (1976) and Agarwala and Dixon (1990).

SEASONAL OCCURRENCE

The overwintering adults emerged from pupae appeared on wormwood and other plants infested by *Macrosiphoniella pseudoartemisiae, Brachycaudus helichrysi* and *Eriosoma lanigerum* between mid and late April, 1984. In the field it was observed that during summer (May and June) the number of different aphids increased on fresh vegetation and initiate the abundance of this predator. This predator has two generations in an year as in *C. septempunctata* (Benham and Muggleton, 1970) in *A. bipunctata* (Pruszyriski and Lipa, 1971). During the monsoon (July-August) when aphid population was scarce in the field, a low number of adults *H. eucharis* dispersed mostly to the surrounding herbs and orchard fields which were infested by other aphids viz. *Phorodon cannabis* (on *Cannabis sativa*) and *Hyalopterus pruni* (on *Prunus persica).* During autumn (September-October) which was a short period after the monsoon in this area, the aphid population started increasing again and was attacking by this beetle. In late autumn the pupae and adults were found to hibernate under the herbs, under the bark of the orchard plants (*Pyrusmalus*) and trees (*Pinus excelsa* and *Populus ciliata*).

The above findings indicate the potentiality of *Harmonia eucharis* as an effective aphidophagous coccinellid to suppress aphids on herb and orchard fields in the area.

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Plumbagin Effects on *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) IV. Final Instar Haemolymph Trehalose, Cations and Nucleic Acids

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Abstract: The effect of a topical ED 50 dose (117.3 µg/g body weight) of plumbagin on haemolymph trehalose, nucleic acids and cation concentrations of final instar larvae of *Helicoverpa armigera* Hubner was studied. Trehalose concentration in the plumbagin treated larvae was significantly lower at 48 hr (45.6%) but was higher significantly at 120 hr (23.4%). Of all the cationic concentrations, K^+ concentration was the least affected by plumbagin treatment. While, Na^+ concentration was significantly higher, the concentrations of Ca^{++} and Mg^{++} were significantly lower in the plumbagin treated larvae. the concentrations of both DNA and RNA were also significantly reduced in plumbagin treated as compared to the control.

Key words: Plumbagin, *Helicoverpa armigera*, Haemolymph, Trehalose, cations, DNA, RNA.

INTRODUCTION

Of late much emphasis is being given to the safety of ecosystem and non-target organisms in the pest management strategies. This can be achieved by the use of effective natural compounds like botanicals in place of synthetic chemicals. Plumbagin (5-hydroxy-2-methyl-1,4 -naphthoquinone) is one such bioactive principle from plants (*Plumbago* spp) reported for its insecticidal, antifeedant and insect growth regulatory properties (Kubo *et al.*, 1980, 1983; Gujar and Mehrotra, 1988; Hassanali and Lwande, 1988 and Chockalingam *et al.*, 1990). It is the first bioactive plant principle known for its chitin synthesis inhibition (Kubo *et al.*, 1983 and Mitchell and Smith, 1988). The limited information on the physiological disturbances due to plumbagin in insects prompted to initiate the present study.

MATERIALS AND METHODS

H. armigera Hub. larvae were maintained in the laboratory under aseptic conditions on a semi-synthetic diet (Paul, 1990) with some modifications at a temperature of 25±1°C, relative humidity of 60±10 per cent and a photoperiod of 16 light and 8 dark hours. Addition of vitamin E and cholesterol were the modifications to the recipe of Paul (1990) that improved larval feeding and pupation.

Final (5th) instar larvae were separated on the basis of their head capsule width within one hour of their moult. They were individually weighed with an accuracy of 1 mg (Mettler PE360). the effective dose 50 (ED_{50}) of plumbagin used in the present study was a predetermined dose that inhibited pupation in the 50 per cent population of the test larvae. Plumbagin was dissolved in acetone and its ED_{50} (117.3 µg/g body weight) was applied topically with the help of Hamilton syringe (10 µl) on the thoracic region of the larvae. Control larvae received only acetone (2 µl) as topical application.

The observations were recorded at 24 hr interval, starting from 0 to 120 hr after the treatment. Since the larvae were treated within 1 hr of the moult to final instar, the initial estimations at 0 hr were considered the same for both control and plumbagin-treated.

Estimation of haemolymph trehalose, cations and nucleic acids.

The haemolymph was drawn by pricking one of the prolegs of the larva with a sterilized needle and collected on a piece of parafilm

membrane kept on ice. Equal quantity of haemolymph from five larvae was pooled into an eppendorf vial which constituted a replication. Minimum of three such samples were collected. The samples were diluted with equal volume of extraction buffer (0.01 M sodium phosphate buffer, pH 7.2; containing 0.05 M potassium chloride, 5 mM ethylene diaminetetracetic acid, 1 mM phenyl methyl sulfonyl flouride and 1 mM phenylthiourea). Each sample was centrifuged at 10,000 rpm (4°C) for 10 minutes using angle set rotar (Kontron; As 24) to precipitate the haemocytes and to get clear plasma.

Plasma trehalose was estimated by anthrone reagent method as detailed by Wyatt and Kalf (1967) using trehalose (BDH-England) as standard.

Plasma without addition of buffer was used for estimation of various cations. All the four cations, sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and magnesium (Mg^{++}) were estimated from the same sample of plasma. The Na^+ and K^+ concentrations were determined by flame photometry, whereas the Ca^{++} and Mg^{++} concentrations were by atomic absorption spectrophotometry (Willis, 1960a, b, c). For Na^+ and Ca^{++} the plasma was diluted 100 times with distilled water and NaCl and $CaCO_3$ served as standards respectively. Whereas for K^+ and Mg^{++}, the plasma was diluted 300 times with distilled water and KCl and $MgSo_4$ served as standards respectively.

Whole haemolymph was used for the estimation of nucleic acids. the method of haemolymph ribonucleic acid (RNA) content was basically that of Schmidt-Thannhauser as modified by Munro and Fleck (1966). Deoxyribonucleic acid (DNA) was extracted and estimated after RNA extraction from the sample according to Sambrook *et al.*, (1989).

The data was analysed by factorial-completely randamized design (Gomez and Gomez, 1984).

RESULTS

Effect on trehalose

The pattern of changes in haemolymph trehalose concentration was the same both in plumbagin-treated and control larvae barring for lower values in the treated. The concentration decreased from 0 to 24 hr in both followed by an increase reaching the highest concentration at 96 hr. There was no significant difference between the mean values of both control and treated (Table 1).

Table 1. Effect of plumbagin on haemolymph trehalose of the final instar larvae of *H. armigera* Hub.

Hours after treatment	Haemolymph trehalose (mg/ml)	
	Control	Treated
0	2.775^{bc}±0.135	2.775^{bc}±0.135
24	0.495^{a}±0.032	0.528^{a}±0.016
48	3.102^{c}±0.276	1.688^{b}±0.123
72	8.604^{d}±0.844	8.145^{d}±0.873
96	14.719^{g}±0.720	13.156^{f}±1.680
120	9.014^{d}±0.677	11.120^{e}±0.969
Mean	6.452^{1}	6.235^{1}
Test	Treatments	Interaction
F	ns	**
LSD 0.05	-	1.136
0.01	-	1.603

± S.D.: n=20 larvae values having a common alphabet/number are not significantly different at the 5% level.

Effect on cations

The pattern of haemolymph Na^+ concentration both in control and plumbagin-treated larvae was the same but for the higher values in treated throughout (Fig. 1a). However, the pattern in both Ca^{++} and Mg^{++} was the reverse with lower values in treated (Fig. 1c and 1d). The pattern with reference to K^+ was a mixed one with lower values in treated till 48 hr followed by higher values till 120 hr (Fig. 1b).

Effect on nucleic acids

The pattern of haemolymph DNA concentration was the same for both control and plumbagin trated larvae till 48 hr but subsequently it decreased in treated in contrast to increase in control (Table 2).

Table 2. Effect of Plumbagin on haemolymph DNA of the final instar larvae of *H. armigera* Hub.

Hours After Treatment	Haemolymph DNA (µg/ml)	
	Control	Treatment
0	$321.67^{c}\pm10.27$	$321.67^{c}\pm10.27$
24	$258.33^{b}\pm13.12$	$248.33^{ab}\pm10.27$
48	$320.00^{c}\pm14.72$	$321.67^{c}\pm8.50$
72	$503.33^{d}\pm12.47$	$233.33^{ab}\pm14.34$
96	$526.67^{d}\pm12.47$	$241.67^{ab}\pm14.34$
120	$521.67^{d}\pm14.34$	$223.33^{a}\pm12.47$
Mean	408.61^{1}	265.00^{2}
Test	Treatments	Interaction
F	**	**
LSD 0.05	10	25.70
0.01	14.21	34.82

±S.D.: n=15 larvae; Values having a common alphabet/number are not significantly different at the 5% level.

The values of haemolymph DNA at 72, 96 and 120 hr in treated were significantly lower. More than 50 per cent reduction in DNA concentration was recorded during these periods. The maximum reduction was 57.2% at 120 hr.

The decreasing trend in haemolymph RNA concentration was almost the same both in control and plumbagin treated till 72 hr. Thereafter the decrease continued in plumbagin treated till 120 hr in contrast to the increase in control. The haemolymph RNA concentration in plumbagin treated larvae was significantly lower throughout (Table 3).

DISCUSSION

Trehalose is the most characteristic and major carbohydrate present in the insect haemolymph. The concentration of trehalose in the insect haemolymph at a point of time depends on its rate of synthesis and utilization (Coutchie and Crow, 1979). The mean trehalose concentration (6.452 mg/ml) in the final instar control larva of *H. armigera* was comparable to that of *Spodoptera litura* larvae (Ayyangar and Rao, 1990). Reduced feeding soon after the mould and utilization of reserve trehalose for chitin synthesis might have led to low trehalose level at 24 hr. During the active feeding stage of the larva (24-96 hr) the trehalose levels are higher suggesting its synthesis from its monosacharide precursors derived from digested food. Further, decline of the concentration during burrowing and prepupal stage (96-120 hr) could be due to higher energy requirement at larval-pupal moult, which must be derived from endogenous sources (Pant and Agarwal, 1965).

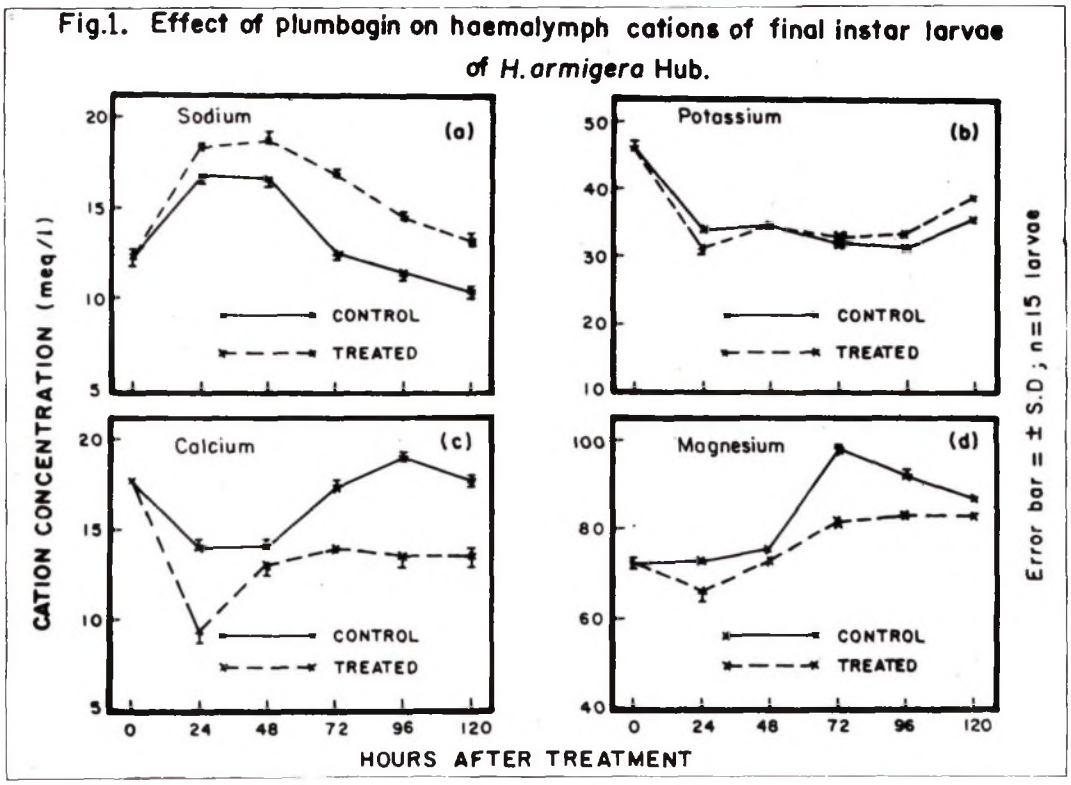
Table 3. Effect of Plumbagin on haemolymph RNA of the final instar larvae of *H. armigera* Hub.

Hours After Treatment	Haemolymph RNA (µg/ml)	
	Control	Treated
0	$320.24^{f}\pm8.35$	$320.24^{f}\pm8.35$
24	$308.75^{f}\pm7.80$	$233.36^{c}\pm5.99$
48	$282.71^{e}\pm7.83$	$261.08^{d}\pm6.63$
72	$221.52^{c}\pm6.63$	$200.56^{b}\pm6.94$
96	$293.87^{e}\pm6.64$	$193.54^{b}\pm8.87$
120	$316.19^{f}\pm6.69$	$145.45^{a}\pm6.80$
Mean	290.55^{1}	225.70^{2}
Test	Treatments	Interaction
F	**	**
LSD 0.05	5.06	12.40
0.01	6.85	16.81

±S.D.: n=15 larvae; Values having a common alphabet/number are not significantly different at the 5% level.

The same is evident from the lower overall mean RNA concentration (225.70 µg/ml) in the plumbagin-treated compared to that of control (290.55 ug/ml). Maximum per cent reduction (41.0%) in plumbagin-treated was recorded at 120 hr.

The lower haemolymph trehalose concentrations till 96 hr in plumbagin-treated larvae could be mainly due to inactive feeding. The effect of starvation on haemolymph trehalose is rather well established (Horie, 1961). The reason for higher trehalose concentration in plumbagin-treated at 120 hr may be due to the extension of the larval period. Similar reduction and delayed build up of haemolymph trehalose concentrations were reported after the treatment of diflubenzuron (Subrahmanyam and Rao, 1986a), methoprene (Subrahmanyam and Rao, 1986b) and azadirachtin (Ayyangar and Rao, 1990) in the young ones of *Achaea janata*



Schistocerca gregaria and *S. litura* respectively.

The haemolymph of phytophagous lepidopterans is characterised by high magnesium (Mg^{++}) and potassium (K^+) and low soidum (Na^+) concentrations (Florkin and jeuniaux, 1974). The mean concentrations of Na^+ (13.39 meq/1) and K^+ (35.68 meq/l) in the final larval haemolymph of *H. armigera* are comparable to that of Na^+ in *H. zea* and Na^+ and K^+ in *H. virescens* (Bindokas and Adams, 1988). However, the Ca^{++} (16.63 meq/l) and Mg^{++} (83.17 meq/1) concentrations were far higher in *H. armigera* larvae. These differences may be either due to species specificity of variation in the diets. The mean ratios of Na^+/K^+ and Ca^{++}/Mg^{++} in the haemolymph of *H. armigera* were less than unity and that of Ca^{++}/Mg^{++} was almost constant as observed in other insects by Clark and Craig (1953).

In the haemolymph of control larvae all the cationic concentrations excepting that of K^+, declined by 120 hr i.e., at prepupal period. Similar decrease in Mg^{++} and increase in K^+ concentration was attributed to reduced haemolymph volume due to gut purge during larval-pupal development (Shimizu, 1982). This was also related to histolysis of goblet cells that regulate the K^+-pump across the midgut during this period (Cloffi and Harvey, 1981). The fall in Mg^{++} concentration in *Bombyx mori* was related to its accumulation in the midgut epithelium as an inorganic phosphate (Kobayashi, 1978). the general fall in Na^+ concentration during the last larval instar can be attributed to the cessation of feeding. The pattern of Ca^{++} concentration may be due to the effect of hormones. As the role of Ca^{++} particularly in the synthesis of JH is well established (Smith *et al.*, 1984 and Kikukawa *et al.*, 1987), its pattern may have relevance with hormonal titres.

Of all the cations observed, K^+ was the least affected by plumbagin. However, the higher K^+ concentration from 48 hr onwards in

the plumbagin treated may be attributed to the effect of plumbagin on K^+-pump. Plumbagin also affected Ca^{++} and Mg^{++} concentrations significantly. Since these cations take active part in important physiological events (Jungries *et al.*, 1974; Smith *et al.*, 1984 and Kikukawa *et al.*, 1987), plumbagin effect on them has greater bearing on the general physiology of the insect. Plumbagin falls in line with other IGR compounds like diflubenzuron (Thomson and Sikovowski, 1982; Subrahmanyam and Rao, 1986a) and azadirachtin (Ayyangar and Rao, 1990) in disturbing the cationic milieu of insect haemolymph.

DNA and RNA are macromolecules concerned with transfer of genetic information and occur in other parts of cells besides, nuclei. The variation in haemocyte DNA concentraion was observed to be dependent on changing phases of moult cycle but no such relationship was observed with RNA concentration (Krishnakumaran *et al.*, 1965, 1967 and Berry *et al.*, 1967). The present results are in accordance with the above observations. Plumbagin treatment decreased both DNA and RNA concentraions of final instar larval haemolymph of *H. armigera*. The decrease in concentrations of DNA could be either due to the direct effect of plumbagin on the DNA synthesis in haemocytes or indirect effect through suppressed ecdysone titre. Similar decrease in nucleic acid concentrations were also reported after the application of IGR compounds like, diflubenzuron (Mitlin *et al.*, 1977 and Deloach *et al.*, 1981) and azadirachtin (Qadri and Narasaiah, 1979 and Shashi Gupta, 1988) from different insects.

Thus, the effect of plumbagin on the haemolymph trehalose, cations and nucleic acids may be due to the lesser feeding of the larvae and the interference of the plumbagin in the hormonal system of the insect.

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A New Species of *Eurytermes* Wasmann (Isoptera: Termitidae) from India

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Abstract: A new species of the termite genus *Eurytermes* is described here from Mandal, Bhilwara, Rajasthan, India. The species is based on soldier and worker castes and is close to *E. buddha*, Bose and Maiti.

Key words: *Eurytermes mohana*, Termite.

INTRODUCTION

Genus *Eurytermes* Wasmann (1902) is purely an Indian genus. Roonwal and Chhotani (1966) have studied and revised the genus thoroughly and have recorded 5 species and subspecies, from India and Ceylon. Subsequently Bose and Maiti (1966) reported a new species from South India. I have come across a new species of the genus from Southern Rajasthan and describe it hereunder.

In addition to the usual categories of types, I have used the type-categories 'morphotype' and 'paramorphotype' to designate specimens of the various castes as they are widely used in social insects where caste polymorphism is prevalent.

DESCRIPTION

1. Imago:

Unknown. *Eurytermes mohana* sp. n.

2. Soldier:

(Text-Fig.1, Table 1):- General: Head-capsule pale yellow to brown; antennae, labrum, thorax, legs and abdomen whitish; mandibles brown, paler basally. Head-capsule fairly and body moderately densely, pilose. Total body length (with mandibles, but without antennae) 5.18-5.87 mm.

Table 1. Body measurements (in mm.) and indices of 4 soldiers of *Eurytermes mohana* sp. n. Rathore

Body-Patis	*Eurytermes mohana* sp. n.	Hol o-type
1. Total body length	5.71-5.87	5.18
2. Head length upto tip of mandibles	2.81-2.93	2.72
3. Length of head to lateral base of mandibles	1.88-2.06	1.88
4. Length of head-capsule to base of antennal socket	1.62-1.75	1.68
5. Maximum width of head	1.18-1.25	1.18
6. Maximum height of head	0.93-1.0	0.94
7. Head Index I (Width/Length up to base of mandibles)	0.60-0.66	0.62
8. Head Index II (Height/Width)	0.77-0.80	0.79
9. Head Index III (Height/Length)	0.48-0.51	0.50
10. Head Index IV (Width/Length upto antenna)	0.71-0.77	0.70
11. Maximum length of labrum	0.15-0.17	0.70
12. Maximum width of labrum	0.28-0.30	0.28
13. Length of mandibles (from upper base of condyles to tip)		
(a) Left mandible	0.87-0.95	0.95
(b) Right mandible	0.87-0.93	0.93
14. Maximum width of left mandible at base	0.43	0.43
15. Left mandibular tooth distance (base of tooth to tip of mandible)	0.25	0.25
16. Right mandibular tooth distance (base of tooth to tip of mandible	0.36-0.37	0.37
17. Head mandibular length index (Left mandible length/Head-Length)	0.42-0.49	0.50

18. Left mandibular index (Width/Length)	0.53-0.57	0.52
19. Left mandibular tooth index (Tooth distance/mandible length)	0.26-0.28	0.26
20. Right mandibular tooth index (Tooth distance/Mandible Length)	0.38-0.42	0.39
21. Minimum median length of postmentum	1.06-1.20	1.20
22. Maximum width of postmentum	0.34-0.37	0.34
23. Width of postmentum of waist	0.22-0.25	0.22
24. Maximum length of pronotum	0.37-0.43	0.39
25. Maximum width of pronotum	0.72-0.76	0.72
26. Pronotum head index (Pronotum width/Head width)	0.57-0.60	0.61
27. Pronotum index (Length/width)	0.51-0.57	0.54
28. Maximum width of mesonotum	0.56-0.60	0.56
29. Maximum width of metanotum	0.66-0.68	0.66
30. Number of antennal segments		
(a) Right antenna	14	14
(b) Left antenna	14	14

Head:

Head-capsule subrectangular; longer than broad (length to base of mandibles 1.88-2.06 mm, maximum width 1.18-1.25 mm; sides substraight, weakly incurved a little behind middle and slightly converging posteriorly; posterior margin convex. Fontanelle: Indistinct. Eye and ocelli: Absent. Antennae with 14 segments; fairly pilose, proximal segments somewhat less so; segment 1 longest, cylindrical; 2 narrower and cylindrical, about half of 1; 3 a little longer than 2; 4 shortest; 5-9 gradually increasing in size and becoming long and club shaped; 10-13 subsequal and club-shaped.; 14 ovate. Clypeus: Divided into an ante and a postclypeus. Anteclypeus a narrow, apilose strip, maximum width 0.35 and maximum length 0.05 mm. Postclypeus pilose; broader than long (maximum width 0.37 and maximum length 0.08 mm.) Labrum: Subtriangular, with a few long and several short hairs. Mandibles: Thick, stout, broad basally and narrower, pointed and incurved distally. Left mandible with a prominent tooth, lying at about one-third the mandible length from distal tip, forming almost a right angle with the inner margin and a small indentation basally. Right mandible with a prominent tooth, lying a little below one-third of mandible length from tip, and a small indentation basally. Postmentum long, club-shaped with a long waist; broadest at about distal one-third and narrowest a little behind the middle, anterior margin substraight; posterior margin concave.

Thorax: *Pronotum*: Strongly saddle-shaped, much narrower than head; broader than long (maximum length 0.37-0.43 mm; maximum width 0.72-0.76 mm; both anterior and posterior margins without medial notch. Mesonotum and metanotum: The former greatly, and the latter slightly, narrower than pronotum: posterior margins of both without a median incurving or notch. Legs: Moderately long and fairly pilose: fore-legs longer than middle legs, the latter shortest; hind-legs longest; fore-tibia somewhat swollen; apical tibial spurs 3 in fore legs (dorsal spur minute) and 2 each in middle and hind-legs (apical spur formula 3: 2: 2). Tarsi 4-jointed, aerolium absent.

Abdomen: Oblong; moderately densely pilose. Cerci 2-jointed, hairy, 0.07 mm long. Styli absent.

3. Worker Major (Text-Fig. 2; Table 2)

General: Head-capsule, postclypeus and antennae yellowish to brownish yellow: mandibles pale brown, teeth and outer margin dark brown; thorax and legs yellowish white to brownish white; abdominal tergites and sternites transparent but appear black because of intestinal contents showing through. Head fairly and body densely, pilose. Total body length (excluding antennae) 4.80-4.84 mm.

Table 2. Body measurements (in mm.) of *Eurytermes moharta* Sp. nov. (Rathore) Worker Major and Minor

Body-Parts	*E. mohana* sp.nov.worker major Range (3-exs.)	Worker minor Range (3 exs)
1. Total body length (excluding antennae)	4.80-4.84	3.10-3.60
2. Length of head to tip of labrum	1.05-1.12	0.92-0.96
3. Length of head to lateral base of mandibles	0.75-0.77	0.53-0.56
4. Maximum width of head	0.88-0.90	0.75-0.77
5. Maximum height of head	0.47	0.35-0.39
6. Diameter of fontanelle		
(a) Length	0.10	0.08
(b) Width	0.08	0.07
7. Maximum length of postclypeus	0.21-0.23	0.17
8. Maximum width of postclypeus	0.40	0.32-0.34
9. Maximum length of labrum	0.24-0.28	0.15-0.17
10. Maximum width of labrum	0.34-0.36	0.24-0.26
11. Maximum length of pronotum	0.31-0.34	0.28
12. Maximum width of pronotum	0.60-0.68	0.46-0.50
13. Number of antennal segments		
(a) Left antenna	14	14
(b) Right antenna	14	14

Head: Head-capsule subcircular, broader than long (Length to base of mandible 0.75-0.77, maximum width 0.88-0.90 mm.) Fontanelle: Present as a white, translucent, elongate spot, situated in mid-dorsum. Eye and ocelli: Absent. Antennae with 14 segments; fairly pilose: segment 1 longest, cylindrical; 2 narrower and almost half of 1; 3 unequal to or slightly shorter than 2; 4 shorter than 3; 5-9 gradually increasing in size; 10-13 subequal and clubshaped; last (14) ovate and longer than the penultimate one. Clypeus divided to an ante and a postclypeus. Anteclypeus a whitish, apilose strip, medially projecting anteriorly. Postclypeus pilose; broader than long (maximum width 0.40-0.42 mm, maximum length 0.21-0.23 mm). *Labrum*: Broader than long (maximum length 0.24-0.28 mm, maximum width 0.34-0.36 mm); pilose apically. Mandibles: Typically *Eurytermes*-type. Left mandible with an apical and 2 marginal teeth; apical finger-like, Ist marginal subequal and nearer to the apical, and with a long posterior margin; 2nd short, triangular, separated from the Ist by a prominent notch; molar plate large and prominent. Right mandible also with an apical and 2 marginal teeth; apical finger like; Ist marginal smaller and nearer to the apical; 2nd short and with an incurved posterior margin; molar plate also incurved.

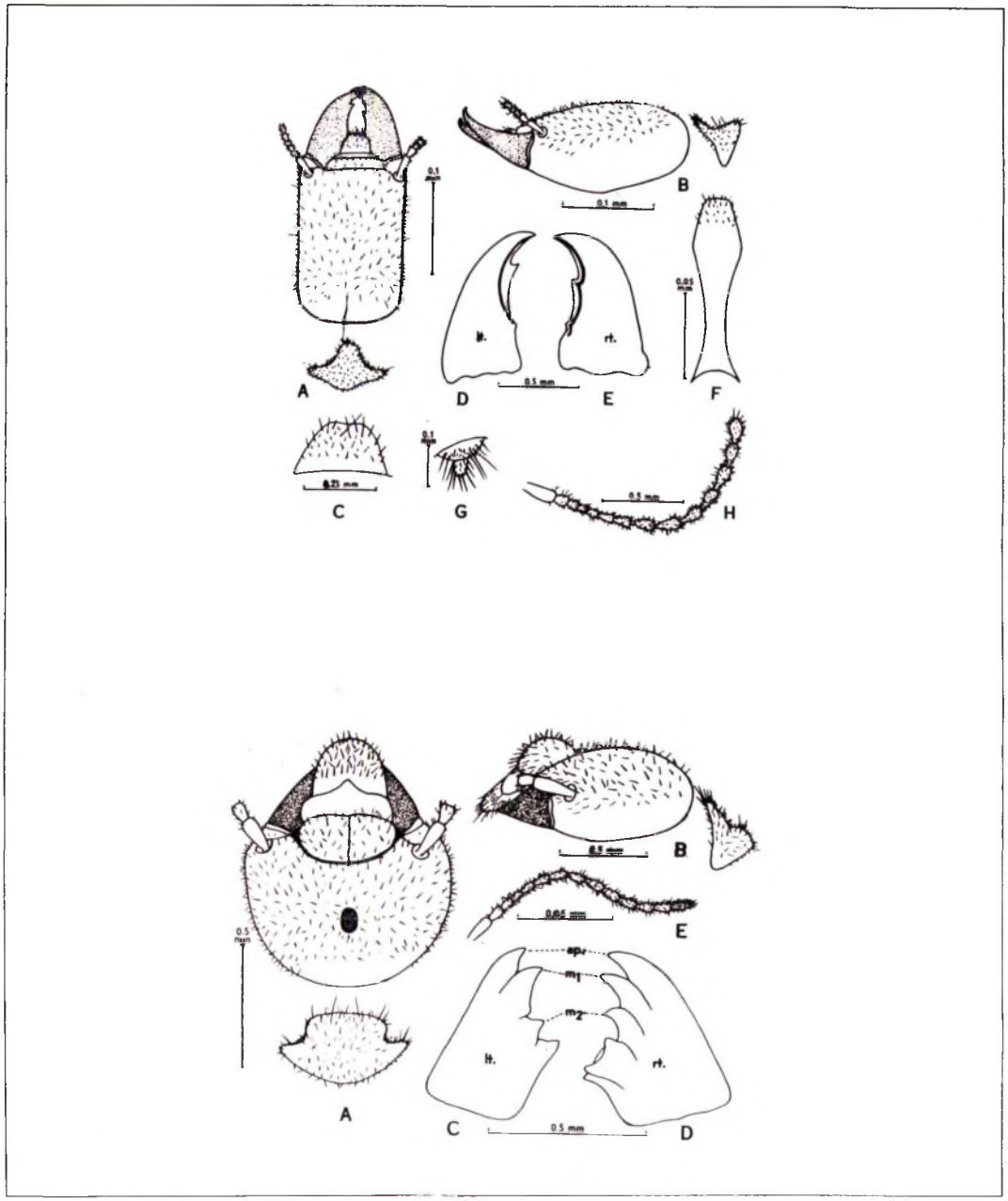
Thorax: *Pronotum*: Strongly saddle-shaped; much narrower than head-capsule; broader than long; both anterior and posterior margins without medial notch. Mesonotum narrower and metanotum almost as wide as pronotum; posterior margin of both without a medial incurving or notch. Legs moderately long and fairly pilose; fore-legs longer than middle legs, the latter shortest; hind-legs longest; apical tibial spur formula 3: 2: 2; tarsi 4-joined.

Abdomen: oblong; moderately pilose; cerci 2-joined; styli absent.

4. Worker Minor

Resembles worker major but smaller in size.

Material: *Holotype*: A soldier in a vial, in spirit; Mandal *Ca* 71 km E. Bhilwara, Rajasthan, India; collector N. S. Rathore, 4.9.1986, ex. 'under stone', deposited in National Zoological Collection, Zoological Survey of India, Calcutta.



Explanation of Text-Figures 1 & 2:

Text-Fig. 1. *Eurytermes mohana* sp. n. Soldier. Holotype from Mands;, Bhilwara district, Rajasthan State, India. (A) Head and thorax in dorsal view (B) Ditto, inside view (c) Labrum in dorsal view (D) Left mandible in dorsal view (Ed) Ditto, right mandible. (F) Postmentum, in ventral view (G) Right carcus in dorsal view (H) Right antenna, in dorsal view. lt., Left mandible., rt. Right mandible.

Text-Fig.2. *Curytermes mohana* sp. n. Worker Major. Morphotype from Mandal, Bhilwara district, Rajasthan State, India. (A) Head and pronotum in dorsal view (B) Ditto, inside view (c) Left Mandible, in dorsal view (D) Ditto, right mandible (E) Right side antenna in dorsal view. lt. left mandible, ap., apical tooth of mandibles., m_1, m_2, Ist and 2nd marginal teeth of mandibles., rt. right mandible.

Paratypes: Morphotype: A worker, in a vial, deposited as above, other data as in holotype. Paratypes (Soldier) and paramorphotypes (workers); Deposited as follows: (a) In Zoological Survey of India, Calcutta: (i) 1 soldier and 2 workers in a vial; other data as in holotype. In Desert Regional Station, Zoological Survey of India, Jodhpur: (i) 1 soldier and 2 workers in a vial, with same particulars as for the holotype.

Type locality and Distribution: Mandal 71 km from Bhilwara, Rajasthan State, India, type-locality. So far known from the type locality.

Comparison: *Eurytermes mohana* is close to *Eurytermes buddha* Bose and Maiti, but differs from it as follows:

Soldier: (i) Head sides substraight, weakly incurved a little behind middle (vs. substraight throughout). (ii) maximum width is more 1.18-1.25 vs 1.03-1.18 mm. (iii) mandibles are longer (Left mandible length 0.87-0.95 vs 0.70-0.85 and Right mandible length o.87-0.93 vs 0.66-0.82). (iv) Left mandibular tooth distance from tip more (0.25 vs 0.19-0.23 mm). (v) Similarly right mandibular tooth distance is more 0.36-0.37 vs 0.21-0.30 mm. (vi) Right mandibular tooth index (i.e., Tooth distance/mandible length) 0.38-0.42 vs 0.35-0.37 (vii) Cerci longer (0.07 mm vs 0.04 mm).

Worker: (1) Major and minor worker present v/s only one type of workers.

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Histology and Secretory Activity of Accessory Reproductive Organs in Male *Opisina arenosella* Walker (Lepidoptera: Xyloryctinae)

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Abstract: Accessory reprouctive organs in male *Opisina arenosella* liberate their secretion by apocrine method. Accessory gland and primary ejaculatory duct unpaired are divisible into three zones based on their secretion. Chemical nature of the accessory reproductive gland secretion is studied by histochemical, biochemical and electrophoretic methods. Protein, glycogen lipid and phenol have been detected while lipoprotein and glycoprotein are absent. Accessory reproductive gland of 0-day adult contains 16 protein bands. Electrophoresis of the gland during development indicates that there is no change in number and position of the peptide patterns in the secretion.

Key words: *Opisina arenosella*, accessory reproductive organs, chemical nature of secretion, electrophoresis.

INTRODUCTION

Development and structure of accessory reproductive glands (ARGs) in male insects have been reviewed (Leopold, 1976; Chen, 1984; Happ, 1984). Most of the insects liberate their secretion of ARGs either by apocrine method (Riemann and Thorson, 1979) or by apocrine and merocrine means (Brits, 1978; Lai-Folk, 1982). Different zones or regions have been identified in the ARG, on the basis of histology of the secretion (Brits, 1978) or both the nature of the secretion and constriction of the gland (Lai-Fook, 1982). Chemical nature of the male ARG secretion in insects has been studied (Gerber *et al.*, 1971; De-Loof and Lagasse, 1972; Vijayalekshmi and Adiyodi, 1973; Ranganathan *et al.*, 1984). Gillott and Venkatesh (1985) and Sridevi (1987) have reported the protein pattern of the secretion during maturation of the ARG. This paper deals with the structure of the ARG and chemical nature of the ARG secretion in *Opisina arenosella*, a member of subfamily xyloryctinae and a major pest of coconut palm.

MATERIALS AND METHODS

The insects for experiments were taken from the colony maintained in the laboratory as described earlier (Santhosh-Babu and Prabhu, 1987). The dissected out ARG from 4-day pupa and 0-day adult were fixed in aqueous Bouin's fluid. Paraffin sections were cut at 6 µm, stained in Mallory's triple stain in the routine histological manner. The stained sections were observed under a microscope.

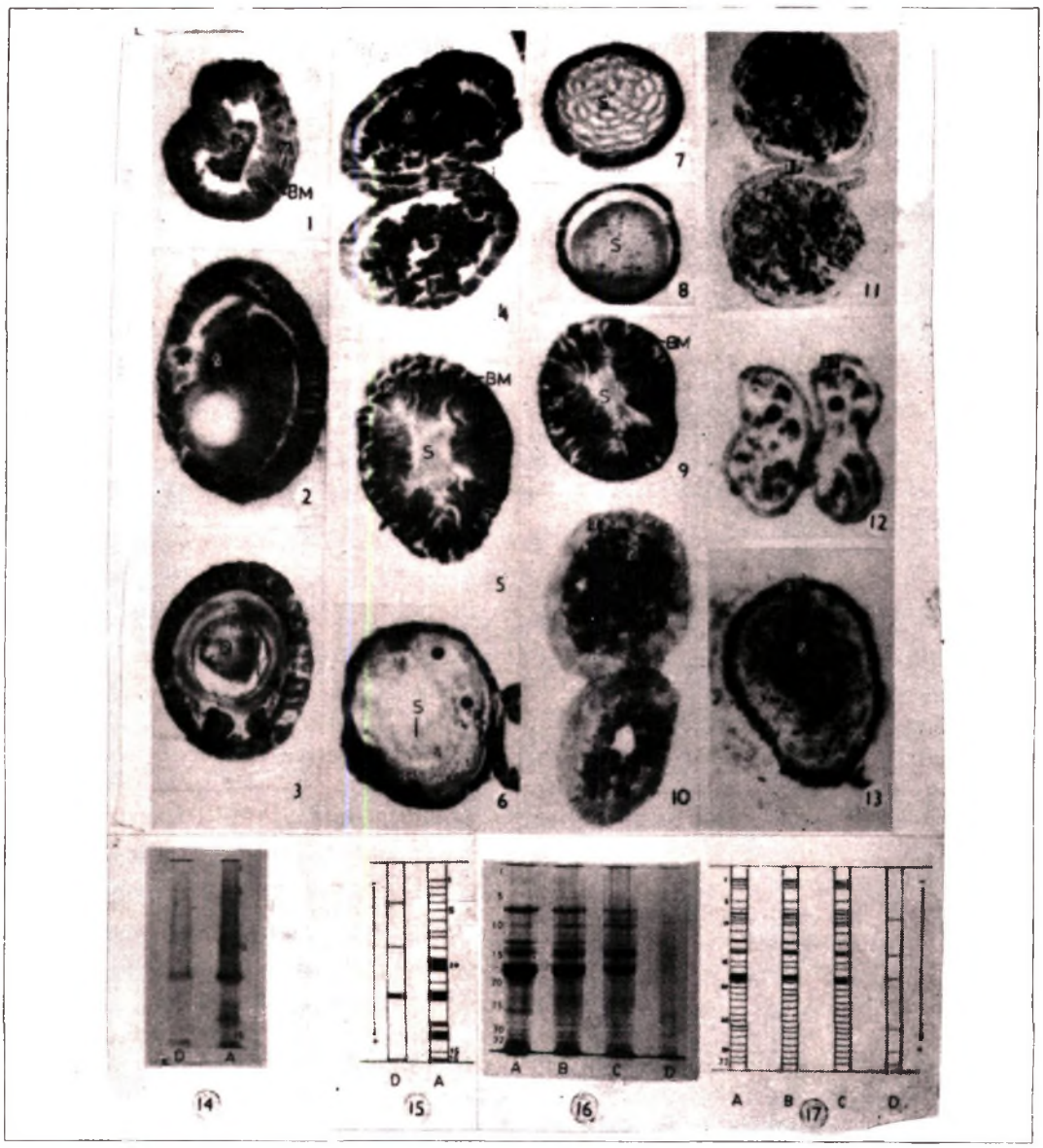
Chemical nature of the ARG secretion

Histochemical:

The dissected out glands were fixed in Carnoy's fixative or 10% formalin, stained in Mercury Bromophenol Blue, or in Million's reaction (Pearse, 1968) for protein. Lipid was stained using Sudan Black B (Pearse, 1968) for which Baker's formaldehyde was the fixative. For the study of glycogen, periodic acid Schiffs reagent (Pearse, 1968) was used as the stain, fixative being picroalcoholic formaldehyde.

Biochemical:

The study was carried out using ARG secretions. For the preparation of secretion the glands were cut into 4 or 5 pieces and put in test tube (3×1 cm). It was kept in ice for 10 minutes and then centrifuged at 6000 g for 5 minutes. The secretion was collected. The procedure of Folin *et al.*, (1969) was followed for protein. Lipid was detected using the methods



Figures 1-9. Transverse sections (stained with Mallory's triple) of accessory reproductive organs of 4-day and 0-day adult showing the secretion in the lumen. Fig. 1. Accessory gland of 4-day pupa showing the release of secretion (S) in apocrine fashion (× 100). Fig. 2. Distal region (zone-1) of accessory gland of 0-day adult (× 100). Fig. 3. Middle region (Zone-2) of accessory gland of 0-day adult (× 100). Fig. 4. Proximal region (Zone-3) of accessory gland of 0-day adult (× 100). Fig. 5. Paired duct of 4-day pupa showing the release of secretion in apocrine fashion (× 100). Fig. 6. Distal region of unpaired duct of 0-day adult (× 100). Fig. 7. Middle region of unpaired duct of 0-day adult (× 100). Fig. 8. Proximal region of unpaired duct of 0-day adult (× 100). Fig. 9. Unpaired duct of 4-day pupa showing the release of secretion in apocrine fashion (× 100). Fig. 10-13. Transverse sections of accessory reproductive organs of 0-day adult showing the secretion (S) BM-Basement membrane, EP-Epithelia, N-Nucleus. Fig. 10. Stained with Mercury bromophenol blue (× 100). Fig. 11. Stained with Million's reaction (× 100). Fig. 12. Stained with PAS (× 100). Fig. 13. Stained with Sudan black B (× 100). Fig. 14-17. Polyacrylamide gel electrophoresis of secretion of male accessory reproductive gland. A-0-day adult, B-8-day pupa, C-6-day pupa, D-4-day pupa. Fig. 14. PAGE (10%)- Non-dissociating gel electrophoresis. Fig. 15. Schematic representation of non-dissociating gel electrophoresis. Fig. 16. SDS-PAGE (10%). Fig. 17. Schematic representation of SDS-PAGE.

of Bragdon (1951). The method of Seifter *et al.*, (1950) was carried out for glycogen. For phenol detection the procedure of Bray and Thorpe (1967) was used.

***Electrophoresis*:**

The method of Bela Takac's (1979) was followed for SDS-PAGE with some modifications. The 1 mm 3% stacking gel (pH 6.8) was followed by a 10 cm 10% separating gel (pH 8.3) with 1% SDS.

ARG from 4,6 and 8-day pupae and 0-day adult were dissected out and their secretions were prepared as mentioned above. The secretions were homogenized, centrifuged and then the supernatant was used as the sample.

Composition of the stacking gel, separating gel, electrode buffer and pH in non-dissociating gel electrophoresis were the same as the SDS-PAGE except in contained no SDS. The dissection and the preparation of secretion were carried out in ice cold medium.

The gel was stained for peptides and proteins by the method of Bela Takac's (1979). Glycoprotein staining was carried out with periodic acid Schiff's (PAS) reagent (Zacharius *et al.*, 1969) and lipoprotein with saturated Sudan Black B (Whitemore and Gilbert, 1974).

RESULTS

Histology: ARGs–accessory glands and primary ejaculatory duct paired and unpaired consist of a single layer of secretory cells. A basement membrane surrounds the epithelial layer. No muscle layers are observed in the ARGs.

The secretion of the cell is discharged into the lumen of the accessory gland in the form of small droplets or granules in apocrine fashion (fig.1). Individual cell boundary cannot be distinguished. Cytoplasm is granular in nature. On the basis of difference in the appearance of secretion, the gland is divisible into three zones. Zone-1, the distal region of the gland contains many small granules which stain light yellowish with Mallory's triple stain (fig.2). In zone-II, the central portion of the lumen is filled with small vesicle, light pinkish in colour. Surrounding the vesicles are small granules appearing in reddish brown colour (fig.3). The other type of secretion released in the proximal zone (III) consists of many large vesicles which stain purple red (fig.4).

The secretion of primary ejaculatory duct paired consists of small granules, reddish brown in colour with the stain. The secretion is liberated in apocrine method (fig.5). Three types of secretions are distinguishable histologically by their appearance in different parts of the lumen of the primary ejaculatory duct unpaired. There is no demarcation or constriction between these regions. The secretion in the distal region consists of small granules, light yellowish in colour (fig.6). In the second region, the secretions are small granules, appearing as net like and stain orange red (fig.7). The third region at the proximal end contains small droplet-like secretion, reddish colour in the periphery while in the middle region its colour is light greenish (fig.8). The secretion is discharged into the lumen in apocrine means (fig.9).

Chemical nature of the ARG secretion:

Some of the globules or granules of the secretion throughout the gland are stained intensely with Mercury bromophenol blue indicating the presence of protein (fig. 10). Million's method also shows highly reddish pink colour in some of the granules or globules along the entire length of the gland (fig. 11). This reveals that the secretion contains protein. The granular epithelia also stain with Million's but the intensity of colour is less when compared with luminal contents. Some of the secretory granules are stained in purplish red by the Schiff's reagent and its presence is seen throughout the gland indicating the presence of glycogen in the secretion (fig.12). Black colour is developed intensely with Sudan Black B in some of the secretory globules of the gland (fig.13). Quantitative studies show the presence of protein, lipid, glycogen and phenol in the secretion of ARG.

Four protein bands-4, 9, 12 and 16 are distinguishable in the secretion of 4-day pupa, among which bands 12 and 16 are more intensely stained (figs. 14 & 15). The width of the band 12 is greater when compared with band 16. In 0-day adult, sixteen protein bands numbered all 1-16 serially are noticed in the secretion. The intensity of the bands 12 and 16 has increased compared to the 4-day pupa. Band 4, 9, 12 and 16 in 0-day adult correspond with the position of band 4, 9, 12 and 16 in 4-day pupa.

SDS-PAGE of the ARG secretion during development:

Six faint polypeptide bands-8, 14, 17, 26, 30 and 32 are distinguishable in the secretion of 4-day pupa (figs. 16 & 17). In 6-day pupa thirty two bands are noted among which bands 7, 9, 13, 14, 17 and 18 are more intense. The bands 8, 14, 17, 26, 30 and 32 correspond with six bands in 4-day pupa and their intensity increases when compared to bands in 4-day pupa. Thirthy two bands (as in 6-day pupa) are also observed in 8-day pupa. The intensity of bands 17 and 18 increases in 8-day pupa compared to 6-day pupa.In 0-day adult the total number of bands (32) are same in the secretion. Bands 7, 12, 13 and 14 are more intense than in 8-day pupa. Both intensity and width of the bands 17 and 18 are greater now.

DISCUSSION

The present investigation reveals that in *Opisina arenosella* ARGs consists of a single layer of columnar cells which liberate their products in apocrine methods as in *Anagasta kuhniella* (Riemann and Thorson, 1979). Lai-Fook (1982) has noticed both apocrine and merocrine fashions in *Calpodes ethlius*. No muscle layers are observed around the epithelium in the present species similar to that reported for *Phthorimaea operculella* (Brits, 1978). On the basis of difference in the appearance of secretion, three regions are distinguished in the lumen of the accessory gland in *O. arenosella*. Light microscopic studies reveal four regions in *Phthorimaea operculella* (Brits, 1978) while in *Anagasta kuhniella* (Riemann and Thorson, 1979) and *Calpodes ethlius* (Lai-Fook, 1982) the recognizable regions are five based on electron microscopic studies. The present observations in *O. arenosella* show three secretory zones in the primary ejaculatory duct unpaired on the basis of staining and appearance of the secretory materials as in *Phthorimaea operculella* (Brits, 1978).

ARG secretion of *O. arenosella* contains protein as in *Leptinotarsa decemlineata* (De-Loof and Lagasse, 1972), glycogen as reported for *Aspongopus janus* (Ranganathan, *et al.*, 1984) and lipid as noticed in *Periplaneta americana* (Vijayalekshmi and Adiyodi, 1973).

In *O. arenosella* ARG secretion of 0-day adult consists of sixteen protein bands, while thirty two polypeptide fractions are noticed when SDS-PAGE is carried out. Sridevi (1987) identified forty nine to fifthy polypeptide bands in the ARG secretion of *Spodoptera litura*. SDS-PAGE studies in *Drosophila melanogaster* show that forty polypeptide fractions are present in the secretion of ARG (Stumm-Zollinger and Chen, 1985). The present study reveals that in *O. arenosella* no lipoprotein or glycoprotein bands are noticed in the gel indicating that lipo-and glycoproteins are absent in the secretion as in *Spodoptera litura* (Sridevi, 1987).

There is no change in number and position of the peptide patterns in the secretion during the development of ARG in *O. arenosella*. This observation reveals that the intensity of proteins increases during development from 4-day pupa to 0-day adult; some protein increase in concentration considerably more than others. In *Spodoptera litura* the ARG during various developmental stages shows that the protein patterns remain unaltered, although the total amount of protein may vary at specific times during maturation (Sridevi, 1987).

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Five New Species of *Macrocheles* (Macrochelidae: Acari) Associated with Scarabaeid Beetles (Scarabaeidae: Coleoptera) from Tamil Nadu, India

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Abstract: A survey of mites associated with the scarabaeid beetles in Tamil Nadu, India, revealed the presence of several species of macrochelid mites. Presently five new species of Macrochelids, namely, *Macrocheles coprephorae* sp. nov. *M. onitisae* sp. nov. on *Onitis* sp., *M. philemonae* sp. nov. *M. podophorae* sp. nov. and *M. scarabae* sp. nov. on *onitis philemon* (F.) are described and illustrated.

Key words: *Macrocheles*, Scarabaeidae

INTRODUCTION

An extensive survey was conducted in Tamil Nadu, to study the mites associated with insects. During the collections, a large number of scarabaeid beetles were screened for mites. Almost all scarabaeid beetles examined harboured mites of different groups in a live condition. Description of five, new species of *Macrocheles* collected from the scarabaeid beetles are given here under.

All measurements given in the descriptions are in microns. The types and paratype slides of the species described have been deposited in the Acarology Collections of the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-641 003, India.

Macrocheles coprephorae sp. nov.

Female: (figs. 1 to 4). Dorsal shield 916 long and 188 wide, with 18 pairs of simple acicular setae, dorsum with reticulate patterns that being more distinct in the posterior region. Poroidotaxy as in the figure (less than 15 pairs): setae jI 18 long, directed anteriorly, parallel, widely spaced and smooth. All other setae on the integument are smooth, other characters of dorsum usual to the genus.

Characters of venter is quite different from other species included here, punctations completely absent but on the ventral shields that shown have simple reticulate pattern. Sternal shield 170 long and 270 wide, with 2 pairs of pores and three pairs of sternal setae of almost equal length (60 long) and simple. The distance between st. 1. and st. 2, 86; and st 2-st. 3 is almost equal 84. The anterior half of the sternal shield faintly reticulate. Epigynial shield longer (380 long) than wide (190), with pair of long (50) simple setae; posterior of the epigynial shield is reticulate and without any shiny punctations. Metasternal plates are oval, with a pair of pores and a pair of simple setae (44 long), ventrianal shield longer (320) than wide (250). The anterior margin is almost straight and tapper posteriorly. The ventrianal shield with two pairs of simple setae, 50 long, a pair of paranal setae, 40 long and a median adanal seta, 46 long, and also with prominent reticulation. A pair of small metapodal sclerites also present. The soft integumental region is characteristically striated.

Gnathosoma-measures 380 long and 190 wide; well sclerotized; all other characters typical to the genus.

Legs: Coxae I to IV bear a stout and short spur; all leg setae slightly pectinate. Legs I to IV measure 752, 695, 728 and 831 long, respectively. Leg chaetotaxy; coxae; 2, 2, 2, 1:

trochanter: 5, 3, 5, 5; femora: 8, 11, 6, 6; genua: 8, 8, 8, 8: tibiae: 11, 8, 7, 7: tarsi: 33, 20, 15, 15.

Type: A holotype female marked on the slide: India: Tamil Nadu: Coimbatore 10. V. 1988. ex. *Coprus* sp. (coprinae: Scarabaeidae: Coleoptera) Coll: C. Chinniah (No 43/1) two paratype slides with females, collection data same as for type.

Diagnosis: This new species resembles *Macrocheles elimatus*. Berlese (Krantz, 1988) in setation. However it differs from the above species in ventral shields, but all the ventral shields have simple reticulate patterns. The body size also varies besides poroidotaxy and number of dorsal setae.

Relation to the host: These well sclerotized dark brown coloured mites were found attached to the hind leg of the coprinid beetles collected from the light traps. These mites were firmly clinging the setae of hosts by their strong and dentate chelae and hence they are phoretic on the beetle.

Macrocheles onitisae sp. nov.

Female (Figs 5 to 8) Length 625 (from tip of corniculi to posterior margin of idiosoma). Dorsal shield 584 long with 25 pairs of setae and 18 pairs of pores (lyrifissures and glands), without distinct procurved line, broadly covering the dorsal shield. All dorsal setae simple, smooth and acicular, setae j1 directed anteriorly, parallel, closely space, smooth and acicular. All setae on the dorsum and on the soft integument are simple and acicular.

Sternal shield 146 long; with three pairs of long smooth setae and two pairs of pores, with fine background punctations. Sternal shield strongly ornamented. The linea oblique anteriores (1. o.a) not so well developed, running from juncture with linea media transversa (1. m. t) just above the area punctate laterals, anteriorly to linea angulata (1. ang.). The L. o. a. is connected to the straight linea arcuata (1. arc.) by rectangular cells. The depressed area posterior to 1. m. t. is bordered laterally by the linea oblique posterior (1. o. p.), and a fold which include sternal pore-2 which extends upto the area punctate laterals (a. p. 1). The ramus of 1. o. p runs up to sternal setae-3. With in the depression, posterior to 1. m. t. there is a spindle shaped area on either side. The area punctate posteriores (a. p. p.) is more punctate. The area enclosed by 1. o. p. and ramus is also deeply punctate. Meta sternal sclerites narrow with one pair of long smooth acicular setae. Metasternal shield membranous anteriorly with little ornamentation and fine punctations. One pair of long smooth setae located on the base of epigynial shield on either side. Many fine wavy markings pass through the shield that are more prominent in the anterior region.

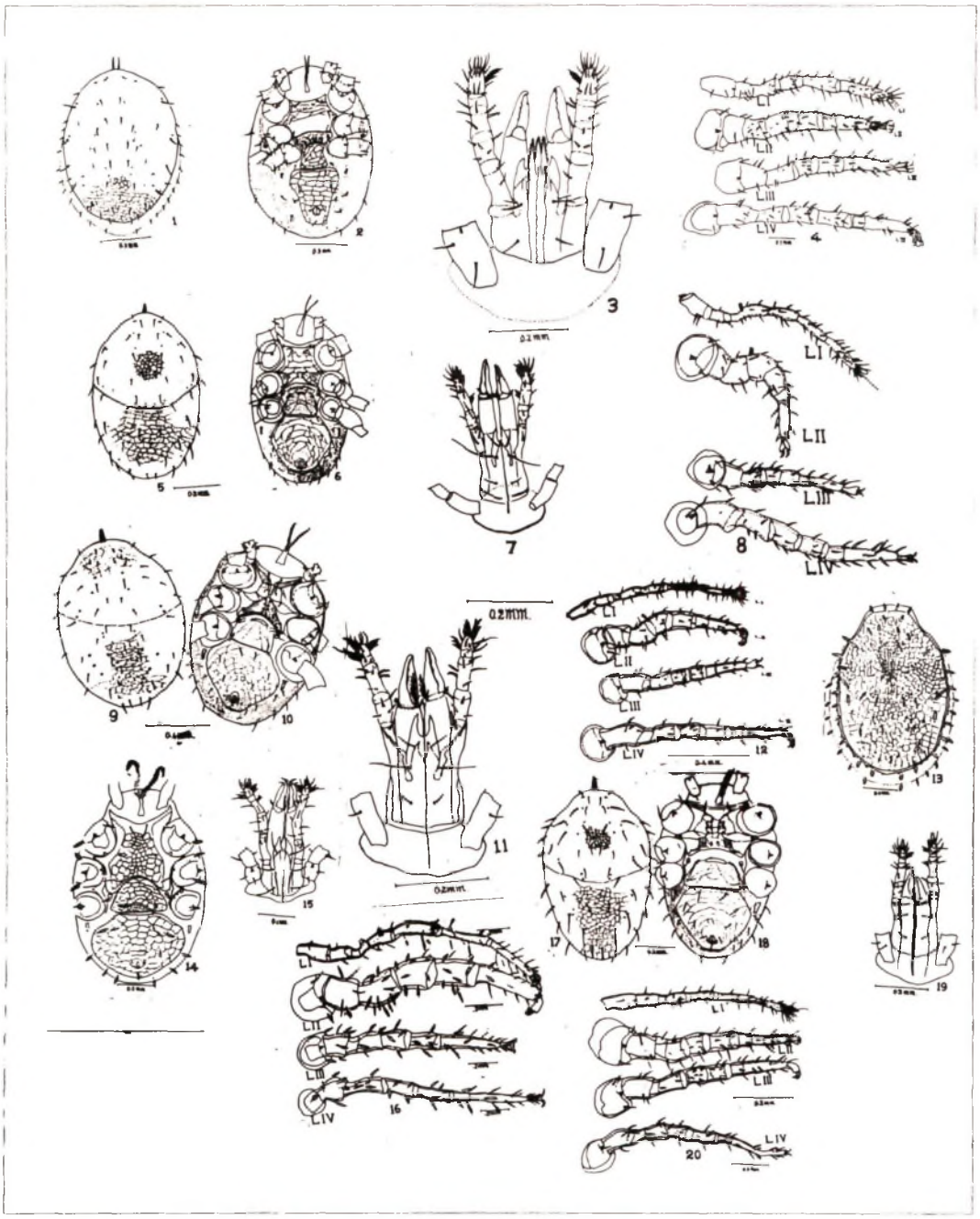
Epigyneal shield 124 long, ventrianal shield longer (203) than wide (188) with a straight anterior margin, punctate-reticulate; with 5 pairs of long smooth ventral setae, a pair of smooth paranal setae and smooth adanal setae (= anal setae). Cribrum is a narrow terminal band present in the area in between the vetrianal shield and the integument with very fine wavy lines that pass around the shield.

Gnathosoma well sclerotized, long, smooth acicular, dutosternal setae one pair hypostomal setae two pairs which are fairly long and smooth. Epistome with a pair of cornicules, chelicerae well sclerotized with toothed chela, with brush-like of filamentous arthrodial process and reduced dorsal setae. The palpal tibia with a pair of strong tines, facing antero laterally and inwards.

Legs: Well sclerotized, coxae II-IV with a strong, stout and short spur each. Leg I without pretarsus. Claws, empodium and other structures absent, but tarsal tip with several setae. Pretarsi II-IV with strong claws. Leg IV is longer than other legs. Leg chaetotaxy: Coxae- 2, 2, 2, 1; Trochanter- 3, 3, 3, 3; Femora, 9, 5, 10, 5: Genua-7, 9, 6, 5; tibiae-8, 5, 3, 5; tarsi 23, 10, 13, 14.

Males: Unknown

Types: A holotype female marked on slide along with seven other females: Tamil Nadu: Coimbatore, 30 v. 1988 ex *Onitis* sp. (Coprinae: Coleoptera), C. Chinniah Coll (41/9). Five paratype slides ♀♀; collection data same as for type.



Figures 1 to 4 *Macrocheles coprephorae* sp. nov. 1. Dorsal view of female; 2. Ventral view of female; 3. Ventral view of gnathosoma; 4. Legs I to IV; Figures 5 to 8 *Macrocheles onitisae* sp. nov. 5. Dorsal view of female; 6. Ventral view of female; 7. Ventra view of gnathosoma; 8. Legs I to IV; Figures 9 to 12 *Machrocheles philemonae* sp. nov. 9. Dorsal view of female; 10. Ventral view of female; 11. Ventral view of gnathosoma; 12. Legs I to IV; Figures 13 to 16 *Macrocheles podophorae* sp. nov. 13. Dorsal view of female; 14. Ventral view of female; 15. Ventral view of gnathosoma; 16. Legs I to IV; Figures 17 to 20 *Macrocheles scarabae* sp. nov. 17. Dorsal view of female; 18. Ventral view of female; 19. Ventral view of gnathosoma; 20. Legs I to IV.

Diagnosis: This new species resembles *Macrocheles caelatus* Berlese (1918) in general structure, ornamentation of sternal shield punctate-reticulate pattern of the ventrianal shield apart from the ventral setal arrangement. The setae Zi/is minute as in *M. caelatus*. The dorsal shield 624 long, setae jI is closely, spaced, parallel and smooth. All dorsal setae are simple, smooth and acicular, L. arc, straight, with not so well developed l. o. a. Five pairs of ventral setae are present on the ventrianal shield. Coxae of legs III and IV bears a straight small spur each.

Relation to the host: The actively moving mites were collected from the body of the black beetles. A few mites were also found attached to the venter of thorax and legs. All mites collected were females, and most had white oval eggs with developing larvae with in the eggs. The egg measured 325 long and 233 broad. Many of the mites were observed attached firmly to the hairs on the ventral side of the thorax of the beetles with the help of chelicerae. Since all collected specimen were only females the relationship between the phoriants and the carrier is a pure form of phoresy.

Macrocheles philemonae sp. nov.

Female: (Figs. 9-12) The dorsal shield 800 long and 530 wide, with 19 pairs of simple, smooth acicular setae and 15 pairs of pores (Lycifissures + glands). Setae jI closely arranged directed anteriorly, smooth. Dorsal shield punctate reticulate without distinct procurved lines. Setae j2 and j3 smaller, smooth and acicular, about 10 long. Setae j5 also short and 20 long. Setae ZI minute and smooth. Setae on the soft integument smooth and acicular. Sternal shield is almost equal in length and breadth, measuring 200 with three pairs of sternal setae and two pairs of pores, strongly ornamented with prominent punctations. The linea oblique anteriores poorly developed, running from linea media transversa (l.m.t) and joining with linea angulata (1. ang.) without joining to linea arcuata (1. arc) that being procurved. The punctations being some what coarse in the depression posterior to l.m.t and the area covered by the l.o.p and the ramus without any punctations. All the sternal setae are smooth long and acicular. The distance between st1 and st2 is 92. The epigynial shield a little broader (160) than long (150) with fine punctation and with a pair of long smooth setae; metasternal shield almost globular either side of the epigynial shield with a long, smooth setae.

Ventrianal shield wider (350) than long, with 3 pairs of smooth acicular ventral setae, ornamentation strong with fine punctuation and rectangular cells. A pair of smooth and simple paranal setae, adanal seta one smooth, cribrum in the form of straight band. A pair of metapodal shield also present. Setae on the soft integument simple and acicular.

Gnathosoma strongly sclerotized and measures 470 long, due to sternal setae one pair, hypostomal setae two pairs long and smooth. Cornicules one pair, very prominent. Chelicerae well sclerotized with toothed chela and a fine brush like arthrodial process. The palpal tarsus bears three branched tines. Legs: The Coxae of the legs I-IV bears a pair of short and stout spurs, one on the upper side and the other on the lower side. Leg I 700 long; without a pretarsus, claws and empodium in the form of tuft of setae. Legs II, III and IV with prominent pretarsi each ending in a strong and stout claw and measure 470, 480 and 880 long respectively.

Leg chaetotaxy: Coxae-2, 2, 2, 0; Trochanter-2, 3, 4, 5; femora-8, 7, 3, 5; genua-9, 7(1), 6, 7; tibiae-10, 6, 5, 4 and tarsi-24, 12, 10, 15,

Types - A holotype female marked on slide along with two other females: India: Tamil Nadu: Coimbatore: 17. iii. 1988. ex. *Onitis philemon* (F.) (Coleoptera). C. Chinniah Coll. No. 20/6; Three paratype slides all with females, collection data same as for holotype.

Diagnosis:

This new species resembles *Macrocheles limue* Samsinak (1962) in the ornamentation of

ventral shield and ventrianal shield but differs in many characters. It is comparatively smaller in size. The dorsal shield measures 800 long and 530 wide with 19 pairs of simple acicular setae. The setae jI are closely arranged, simple and acicular. The sternal shield ornamentation also varies slightly. Instead of two recurved l. arc in *M. limue* only one l. arc is present in the new species without joining to the l. o. a. The palpal tarsi bears three branched tines. The genu of the leg II bears a stout spatulate setae. It also differs from *M. onitisae* sp. nov. described here by the sternal shield ornamentation where in *M. onitisae* the l. arc is straight joining to the l. o. a. whereas in *M.philemonae* l. arc is recurved without joining to l. o. a.

Relationship to the host: The dark brown coloured and heavily sclerotized mites were isolated from the adult beetle *Onitis philemon* (F) collected with light trap. This species was also collected from several other unidentified coprinae beetles. They were found attached to the venter of thorax, antenna and also to the coxae of the hind legs. All collected specimens were females and they invariably had eggs in the opisthosoma with developing larvae within. The egg measured 280 long and 244 wide. The adult mites were observed firmly attached to the hairs on the body of the beetle by their strong chelae and hence the relationship is the simple phoresy.

Macrocheles podophorae sp. nov.

Female (Figs. 13 to 16)- very large sized mite, with interesting features and complex setation, measuring 1739 in length, with 20 pairs of dorsal setae. Setae wither strongly bipectinate, pilosa or simple and smooth. Setae j4 comparatively longer with two rows of prominent bipectinate hairs. Poridotaxy usual, with 11 pairs of pores. Dorsal shield measures 917 long and 611 wide, margin of this shield with strong and short peg-like structure at regular intervals a character that has never been observed in any other species of macrochelids. The dorsal shield ornamentation is strong with almost honey comb-like patterns, that being more clear in the posterior part of dorsal shield.

Sternal shield measure 470 long and 376 wide with characteristic ornamentation, without shiny punctation but with a honey comb-like pattern similar to that on dorsal shield, sternal set are 3 pairs simple, long and acicular, with two pairs of pores. Thus it deviates from other species of macrochelids where l. arc, l. m. t. and other lines are distinct and clear. The distance between st1-st2 and st2-st3 measure 2588 and 188 respectively; epigyneal shield with rectangular cells that being smaller in the anterior region, with a pair of bipectinate setae, and a pair of metasternal sclerites on either side of it with a pair of smooth and simple setae. The epigynal sclerite is 423 wide and 306 long.

Ventrianal shield wider (752) than long (611), with 3 pairs of pectinate ventral setae, and the ornamentation strong with honey comb-like pattern, paranal setae smooth and simple, adanal setae pectinate. Other setae on the integument bipectinate; the tritosternum forked, tritosternal lacina long and hairy.

Gnathosoma measures 940 in length, typical of macrochelides except that palpal; tarsi with laterally pointed, characteristic spatulate setae.

Legs: Legs are very robust and thickly sclerotized, with several types of setae, either bipectinate, pilose, pectinate or simple and smooth acicular setae. Let I without a pretarsus; other legs (II-IV) ends in a pair of strong claws. Coxae of legs I to IV have strong, stout spur, one located on dorsal and the other at the ventral side. Legs I to IV measure 1645, 1363, 1442 and 2162 long, respectively.

Leg chaetotaxy: Coxae; 2, 2, 2, 1; trochanter: 4, 6, 5, 5; femore: 9, 11, 4, 4: Genua: 7, 9, 4, 4: tibiae: 7, 10, 6, 7, and tarsi 24, 16, 16, 14.

Diagnosis:

The new species resembles *M. capensis* (Walter and Krantz 1986) in possessing bipectinate setae on dorsum, venter and legs but differs in many other characters from *M. capensis* and other species included here. The

dorsal shield has 20 pairs of strongly bipectinate setae including setae jl and with honey comb like pattern on the dorsal shield. The sternal shield and ventrianal shield without shiny punctate lines but possess prominent rectangular cells, thus differing from all other species. Besides the margin of dorsal shield has a row of strong stout and short peg like projections.

Types: A holotype female marked on the slide, India: Tamil Nadu: Coimbatore, 17 iii. 1988 ex-*onitis philemon* (F) (Coleoptera) C. Chinniah Coll. (No. 20/1) three paratype slides all with females, collection data same as holotype.

Relationship to the host: Very robust dark brown coloured mites were isolated from the host beetles collected with light traps. The mites were always found clinging to the tarsi and pretarsi of the hind legs invariably by means of the strong claws and chela.

Since all collected specimens were female and from other observations it seems that the relationship is a simple phoresy.

Macrocheles scarabae sp. nov.

Female (figs. 17 to 20). The dorsal shield 916 long and 611 wide, with 21 pairs of dorsal setae, simple smooth long and acicular pores 11 pairs, setae jl smooth, closely arranged and directed anteriorly; dorsal shield punctate-reticulate without distinct procurved lines; setae j4 pilose and present at the posterior part of dorsal shield, all other dorsal setae simple and smooth. Setae Z1 is comparatively smaller (12 long) than all other dorsal setae.

Sternal shield longer (216) than wide (211) with strong ornamentation and prominent punctations; sternal setae 3 pairs linea arcuta (1. arc.) two, procurved the second one almost touching the 1. m. t. The 1. o. a starts from 1. m. t. and joins to the 1. ang. without joining to 1. arc, the region posterior to the 1. m.t. (a. p. p) with a pair of prominent elliptical punctate areas. The area punctiformes is weakly punctated. The distance between St1-St2, St2-St3 measure 611 and 498 respectively. All other characters characteristic of the genus *Macrocheles*. Epigynial shield 211 long and 188 wide with a pair of simple setae, metasternal shield one pair, with long simple setae. Ventrianal shield measures 291 long, and 306 wide with fine semicircular punctations and 3 pairs of simple and smooth ventral setae. Paranal setae one pair; simple and smooth adanal setae present. Cricibum, simple band like ventral setae on the integument smooth and simple. Metapodal shield one pair located on either side of the ventrianal shield.

Gnathosoma: well sclerotized with a pair of smooth and simple deutoseternal setae which measure 400 long. Chelicerae with toothed chela which bears a spur and brush like arthrodial process. Palpal tarsus with three branched tines.

Legs robust and measure 780, 643, 634 and 1034, long respectively. The setae on the legs simple, spine like, peg-like, apart from being acicular, The dorsal setae on fomora-IV pilose. Leg chetotaxy: Coxae: 2, 2, 2, 1; trochanter: 3, 5, 4, 5; Femora: 8, 7, 4, 5; Genua: 8, 7, 8, 7: Tibiae: 8, 8, 7, 7; Tarsi: 22, 19. 16, 15.

Types: A holotype female marked on slide along with another female. India: Tamil Nadu: Coimbatore, 29 iii. 1988; ex-*onitis philemon* (F.) (Coleoptera). C. Chinniah Coll. N. 23/1. Three paratype slides all with females, collection data as for holotype.

Diagonosis: This new species resembles *Macrocheles perigrinus* Krantz (1981) in its general structure and the ornamentation of sternal and ventrianal shields, but differs in many respects. The dorsal shield has 21 pairs of simple setae with 11 pairs of pores: setae jl is not pilose as in *M. periorinus*. In addition, setae j4 is pilose and short while all other dorsal setae are simple and relatively longer. The sternal shield has two procurvel. acr. which do not join 1.o.a. Posterior to the 1. m. t. two elliptical punctate areas (a. p. p.) are more promenade and the area punctiformes is weakly punctate. It differs from *M. onitisae* sp. nov. and *M. philemonae* sp. nov. where the former has a straight 1. arc. on the sternal shield, joining with the 1. o. a. Besides the a. p. p. is spindle like with scattered punctations in both the cases. The dorsal and ventral setae are simple and smooth in both the species while j4 is

pilosa in *Macrocheles scarabae* sp. nov.

Relationship to the host: The dark brown and robust mites isolated from the host *Onitis philemon* collected with light traps were found attached to the venter of thorax, neck, antenna, coxae and several other parts of the host. The adult females containing white hyaline eggs (400 long and 347 wide) and developing larvae within, were found firmly attached to the hairs of the beetle by means of toothed chelae and hence the relationship appeared to be a simple phoresy.

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Legends to Figures

ENTOMON 1995 VOL. 20 NO 3 & 4: 223-227

Relationship Between the Chemical Similarities of Some Compounds and the Similarities in Their Biological Effects on *Galleria mellonella* L. Females

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Abstract: In the present study, type of the biological responses generated by some aliphatic esters on *Galleria mellonella L.* have been correlated with the similarity groups formed by some steric, physicochemical and topological variables of these esters. Observations suggested that as the chemical dissimilarity that can be described by the variables used in the present study to n-nonanal (major component of the natural sex pheromone secreted by the *Galleria mellonella L.* Males) increases, the type and degree of biological response exhibited by *Galleria mellonella L.* females upon exposure to the chemical differs than that of n-nonanal.

Key words: *Galleria mellonella L.* topological indices, steric parameters, physical parameters, attractants, numerical taxonomic method, chemical similarity, phenogram.

INTRODUCTION

Studies based on the use of a reference structure to compare the molecular shapes which are quantitatively or qualitatively correlated with certain activity are known in literature (Hopfinger, 1980; Motoc & Dragomir, 1981).

Shape descriptors developed by many researchers (Amoore, 1967; Allinger, 1972; Simon *et al.*, 1977; Purcell & Teasta, 1977) have been long used for the odour similarity and for some other purposes. All these quantitative structure activity relationship (QSAR) studies seem to offer promising perspectives to model the steric effects in biological systems.

Recently, QSAR studies involving topological indices have been quite popular. The topological indices are certain numerical quantities based on various invariants or characteristics of molecular graphs (Balaban *et al.*, 1983; Gutman & Polansky, 1986).

Males of *Galleria mellonella* (greater wax moth) secrete an odorous substance which is highly attractive to females (Jacobson & Beroza, 1963). Later, this substance has been identified as a combination of n-nonanal and n-undecanal; n-nonanal being as the major component (Leyrer & Monroe, 1973).

n-Nonanal which is an aldehyde is not very stable at atmospheric conditions and easily oxidized to nononoic acid which we found it inactive in bioassays (Turker *et al.*, 1993). Therefore, we had tested the effects of various esters which are more stable at atmospheric conditions on the females of *Galleria mellonella L.* and the results were reported by us previously (Turker *et al.*, 1993).

In the present study, certain steric, physical and topological characteristics of some esters used in the previous study have been evaluated by us in the numerical forms. These esters have been grouped on the basis of the similarities of their above mentioned characteristics by numerical taxonomic methods. Although, numerical taxonomic methods are used primarily for the grouping biological entities with respect to their characteristics, the same methods also have applications in chemosystematics as explained in Sneath and Sokal's book in general (Sneath & Sokal, 1973). To undertake a numerical taxonomic study on the chemical compounds many characters of the compounds,

as many as possible are to be taken into account. All kinds of characters are equally desirable. The values of the characters are standardized and all of the variables are used to determine a matrix which denotes the degrees of similarities between the compounds is obtained. One of the clustering methods is then employed to form the similarity groups of the chemicals. These groups are illustrated by the phenograms which are the useful summarization of the resemblance relationships of the chemical compounds.

In the present study, association between the biological effect of some esters tested on *Galleria mellonella L.* and the similarity groups based on steric, physical and topological variables wee examined.

METHODS

i) Measurements of steric, physical and topological variables of the compounds and their standardization.

In the present work, to characterize the esters, steric, physical and topological variables have been employed. Molar volumes and group volumes, calculated according to the approach of Troube (Wiberg, 1963) have been used as steric variables. For that purpose, the esters were partitioned into two parts as R'-C=0 and -OR. The corresponding volumes are indicated by $V_{R'CO}$ and V_{OR}, respectively. Whereas, the total molar volume is represented as V_T. In addition to molar volumes, the parachore (Glasstone & Lewis, 1970) values (P) were calculated and used as one of the steric variables characterizing the compounds of interest.

The class of physical variables includes the surface tension (γ), molecular weights (M), densities (d) and boiling points (Bp.) of the compounds.

On the other hand, a very detailed analysis of the topology of the compounds has been carried out. Various topological indices were calculated so that the topology class contains thirteen variables. These are the platt index (Platt, 1947, 1952) (F), partial and total Hosoya indices (Balaban *et al.*, 1983; Hosoya, 1971; Narumi & Hosoya, 1980) (Z_i and Z), the partial and total average distance sum connectivity indices (Balaban & Filip, 1984) (J_A, J_B and J).

Since, the numerical values of variables within the same set do not have the same order of magnitude, certain multipliers were used to rescale the magnitudes. Thus steric, physical and topological classes (I-III classes) possess the following set of variables.

Steric variables: (V_{OR}, $V_R{}^{i}{}_{C=0}$, V_T, 0.5(P)) (I)
Physical variables: (4γ, M, 100 d, Bp) (II)
Topological variables: (F, Z_0, Z_i, Z, $100J_A$, $100J_B$, 100J) (III)

By using the method of cosines, state of each of the compounds of interest against n-nonanal (the major component of the sex pheromone of *Galleria mellonella L.)* is determined for each class of variables. Standard values thus found for each class of variables are presented in Table 1.

ii) Construction of the similarity groups of the compounds.

Values of the three classes of variables (I, II, III) are used as the input values of the SIMINT subprogram of NTSYS-PC Program (Wolf, 1992). To indicate the degrees of similarities between the compounds average Euclidean distances between the esters in the 3-dimensional space are calculated by this subprogram. Similarity matrix formed by these distances are subjected to UPGMA (unweighted pair group method using arithmetic averages) algorithm by means of subprogram called SAHN. The output is processed by the subprogram called TREE and the result that is groups constituted by the esters having similar characteristics have been shown in the form of a phenogram (Figure 1).

RESULTS AND DISCUSSION

List of the compounds examined for their steric, physicochemical and topological variables along with the values of these variables are given in Table 1. On the last column of Table 1. effects of these compounds on the females of *Galleria mellonella L.* as was previously reported by Turker *et al.*, (1993) are indicated. It can be seen, that these esters generated three types of responses on the fe-

Table 1. Compounds, standardized values of their variables with respect to n-nonanal and biological responses exhibited by *Galleria mellonella L.* females to these compounds

Compound	Steric (Class I)	Physico chemical (Class II)	Topological (Class III)	Biological response type	Rank of the response a,b
Nonanal	1.00000	.99999	.99999	Active	1
Methyl octanoate	.99507	.99837	.99473	Active	2
Ethyl octanoate	.98885	.99901	.96943	Active	3
Methyl decanoate	.99757	.99691	.89008	Active	
Propyl octanoate	.98115	.99801	.89702	Inactive	
Octyl acetate	.84362	.99878	.95469	Agitative	
Ethyl decanoate	.99326	.99353	.76892	Agitative	
Butyl octanoate	.97272	.99595	.77173	Inactive	
Decyl acetate	.82916	.99641	.75900	Agitative	
Propyl decanoate	.98672	.34954	.61301	Inactive	
Butyl dacanoate	.94230	.34868	.61181	Inactive	
Ethyl nonanoate	.89896	.49831	.45597	Inactive	
Methyl nonanoate	.61047	.34857	.62255	Inactive	
Butyl nonanoate	.84955	.52360	.45576	Inactive	
Propyl nonanoate	.85570	.50014	.45521	Inactive	
Undecanal	.74259	.51662	.45828	Inactive	
Nonanoic acid	.76802	.44588	.46438	Inactive	

(a) 1, 2, 3 are the ranks of the degree of active responses in decreasing order (fewer number of females are attracted by the test chemicals). All three groups of chemicals have significantly different ($P < .05$) effects from each other.
(b) Vertical line indicates that the degree of active response generated by the two chemicals are statistically equivalent.

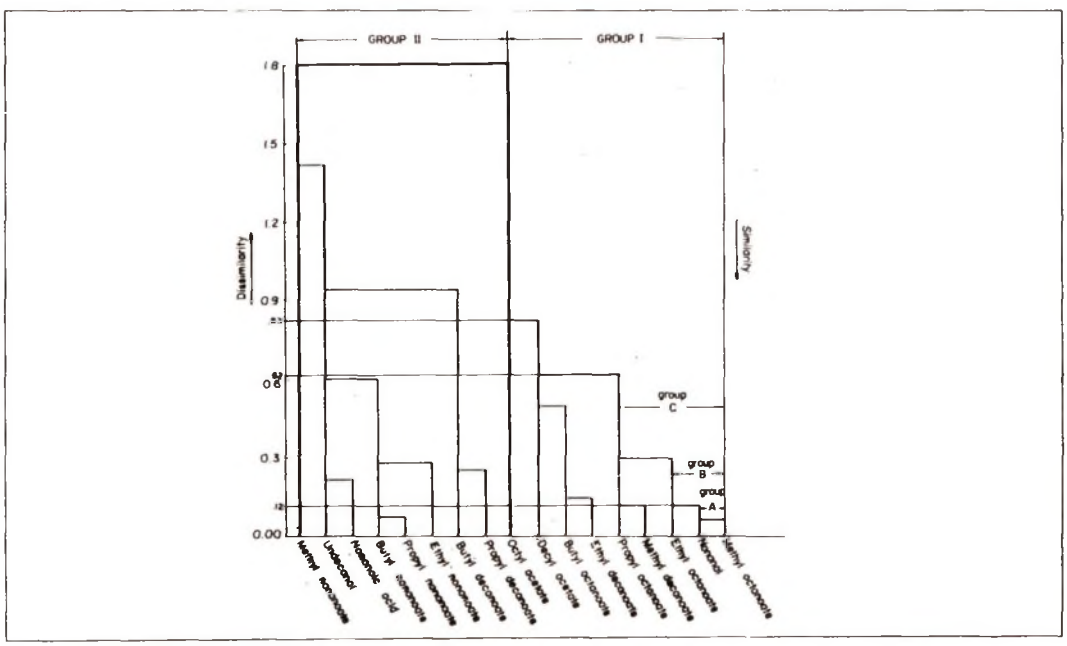

Fig. 1. Phenogram illustrating the similarity groups of the chemical compounds based on their steric physical and topological variables.

males. These are: "Active" type which stands for very similar behaviour of the females to that observed during exposure to n-nonanal, including movement towards the test chemicals; "Agitative" type indicates a high degree of mobility with no indication of being attracted by the chemicals; "Inactive" type as the name indicates, exposed insects to the compounds are sessile. The further details of the responses can be found in the study carried out by Turker *et al.*, (1993).

Among the sixteen compounds tested for their effects on the females, four of these generated "Active" and three of them generated "Agitative" type of responses (Table 1.). Undecanal, is the minor component of the natural pheromone of *Galleria mellonella L.* (Leyrer & Monroe, 1973; Schmidt & Monroe, 1976). Although, the values of the variables are evaluated for this compound (Table 1), biological effect of it was not tested.

When the compounds are grouped with respect to the similarities of their three classes of variables the phenogram given in Figure 1. is obtained. From the phenogram it can be observed that methyl octanoate exhibiting the highest similarity to n-nonanal (major component of the natural pheromone of *Galleria mellonella L.)* forms a group with n-nonanal (group A.). Methyl octanoate is also the compound which generated "Active" response was statistically equivalent to the degree of this response generated by n-nonanal.

Ethyl octanoate is the second chemical which exhibits high degree of similarity to n-nonanal-methyl octanoate pair with respect to steric, physicochemical and topological characters. It is also one of the compounds which causes "Active" response in females but the effect of it is statistically ($P<.05$) less than the effect generated by n-nonanal and methyl octanoate. These results suggest the presence of very similar chemical properties (dissimilarity $\leq .12$) among the methyl octanoate, n-nonanal, ethyl octanoate (members of group B, Figure 1) that can be described in terms of steric, physicochemical and topological variables. At least part of these properties must be essential to invoke the "Active" type of biological response in females of *Galleria mellonella L.*

Methyl decanoate which is the least effective compound among the tested ones generating the "Active" type of response shows high resemblance with propyl octanoate (response type to this compound was "Inactive") in terms of the molecular similarities. However, this last pair again is the closest pair to the methyl octanoate-n-nonanal-ethyl octanoate group (group B.). All four compounds generating "Active" response are in group C (Figure 1.).

As the average chemical dissimilarity to group C increases to .62-.83 for the compounds, as it is for ethyl decanoate, butyl octanoate, decyl acetate and octyl acetate they generally initiate "Agitative" type of response on *Galleria mellonella L.* females. Perhaps, this level of dissimilarity indicates the presence of only a part of the essential chemical properties invoking the "Active" type of biological response in females.

Phenogram shown in Figure 1. indicates that chemicals under consideration are forming two broad groups: Group I containing n-nonanal and Group II containing undecanal as one of their members. Group I holds all of the compounds generating biological responses both "Active" and "Agitative" types. Only two compounds propyl octanoate and butyl octanoate are in this group yet they do not have any effect on the females (at least their effects if there exists any could not be detected with the test conditions that we had, Turker *et al.*, 1993.

The occurrence of propyl and butyl octanoates in Group I may also indicate the following (i) the necessity of higher number of variables that should be used in the present study to increase the sensitivity in identifying biologically important components of the chemical similarities, and (ii) presence of similarities in different respects: for example, methyl decanoate is very similar to propyl octanoate in certain respects but this similarity is not on the chemical property of invoking biological activity.

In Group II, except undecanal, all of the compounds were tested for their biological effects. Females were "Inactive" to these chemicals. Therefore, it can be suggested that as the chemical dissimilarity to Group I. increases for the compounds, as it is for the Group II members (average dissimilarity for these compounds is 1.8), propanility of generating any type of response in females becomes null. In this context it may be suggested that undecanal alone may not have any biological effect on the females. Perhaps, the presence of this compound is important in stabilizing the effect of n-nonanal or together with n-nonanal, undecanal has a synergistic type of biological effect on the females. Generally, those aliphatic esters having similar chemical properties generated similar type of biological responses in *Galleria mellonella L.* females.

When the chemical dissimilarity to n-nonanal (major component of the natural pheromone of *Galleria mellonella L.*) is the lowest, the compounds generate biological response equivalent to that of n-nonanal. As the chemical dissimilarity increases, the type and degree of biological response exhibited by the females upon exposure to the chemical changes considerably.

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A New Species of Genus *Peus* Konow (Hymenoptera: Tenthredinidae) from India and a Revised Key to the Oriental Species

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Abstract: A new species of genus *Peus* konow is described from India, with this, the total number of Indian species of this genus comes upto three. Figures of Male and Female external genitalia are given. A revised key has been provided for all the known species of this genus from the Oriental region.

Key Words: New species, *Peus*, Hymenoptera, Tenthredinidae, India.

INTRODUCTION

Genus *Peus* was erected by konow, 1903, by taking *Peus privus* as its type species. It is represented by 3 species and one subspecies from the Oriental region. Two species *P.privus* Konow, 1903 and *P.pannulosus* Konow, 1907 are known to occur in India, while *P. tibetanus* Malaise, 1945, *P. privus-victoriaemontis* Malaise, 1945 are reported from Tibet and Burma respectively. By describing a new species *P. nefaensis,* sp.nov. total number of Indian species rises to three.

The Holotype is presently in the collection of Authors and will be submitted to IARI, Pusa, New Delhi, after this work is published.

Abbreviations

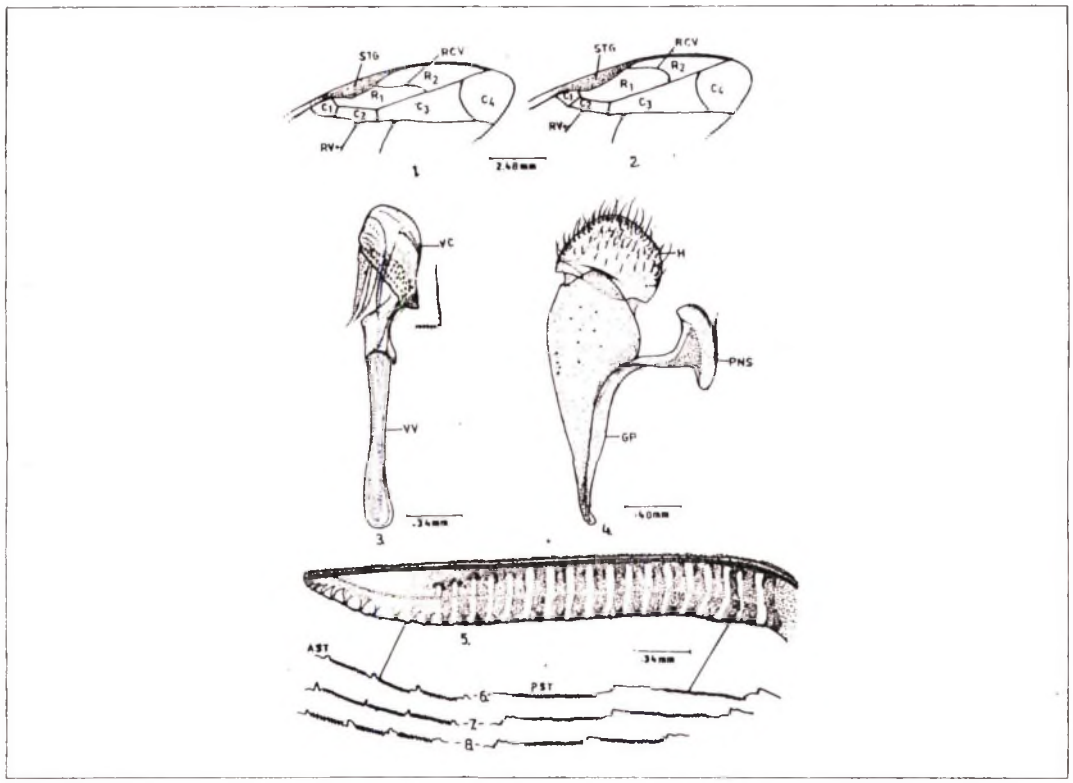
BAS–Basalis; CUB–Cubitus, C_1, C_2, C_3, C_4,–Cubital cells; E1–Eye length; ICD–Intercenchri distance; IDMO–Interocular distance at the level of median ocellus; ITD–Inter-tegular distance; LID–Lower interocular distance; OCL–OCello-occipital line; OOL–Oculo-Ocellar line; POL–Post-ocellar line; AST– Anterior subbasal teeth; PST–Posterior subbasal teeth; RV–Recurrent vein; RCV–Radial crossvein; R_1-R_2–Radial cells, STG–Stigma; VC–Valviceps; VV–Valvura; GP–Gonostipes; H– Harpe; PNS–Parapenis.

Key to the Oriental species of Genus *Peus* Konow

1. Mandibles dark sanguineous, hind-orbits pale yellow; plack are : postocellar area; posterior side of head; a broad band from it to upper hind corner of each eye; four apical antennal segments entirely and remaining flagellar joints with a black ring close to base; ---------- *P.pannulosus* Konow, 1907 In addition to mandibles, hind-orbits; the posterior part of head and postocellar area dark sanguineous; furrows around postocellar area black; 3 apical antennal joints likewise black----------2

2. Head behind eyes evenly and distinctly narrowing in both sexes; face on and around frontal area distinctly and coarsely punctured; maximal height of each supra-antennal tubercle equal to half the length of same tubercle; base of antennae pale yellow; ---------- 3 Head dilated behind eyes in female only; punctures on and around the frontal area ill-defined and indistinct; maximal height of each supra-antennal tubercle only 1/3rd of its length; pale part of antennae fulvous ---------- *P. tibetanus* Malaise, 1945.

3. First recurrent vein joins CUB in the centre of C_2, RCV distinctly and sharply curved (Fig. 1); postocellar area reddish brown; tip of fourth tarsal joint and last joint entirely infuscated in all legs; ratio of height and width of supraantennaltubercles as 1: 2.0; clypeus truncate in male; posterior slope of scutellum punctured, not wrinkled; LID : IDMO = 10:1.5, OOL : POL : OCL = 3.6:1.0:3.2; ratio of antennal segment 1-9 as 2.2 : 1.0 : 4.5 : 3.0 : 2.7 : 2.1 : 1.6 : 1.5 : 2.0; ITD : ICD = 3.6 :



1. Fore wing of *P. privus* konow; 2. Fore wing of *P. nefaensis* sp. nov.; 3. Penis valve of *P. nefaensis* sp. nov.; 4. Gonoforceps of *P. nefaensis* sp. nov.; 5. Lancet of *P. nefaensis* sp. nov.; 6. Detail of anterior and posterior subbasal teeth of *P. nefaensis* sp. nov.; 7. Detail of anterior and posterior sub basal teeth of *P. pannulosus* Konow; 8. Detail of anterior and posterior subbasal teeth of *P. privus* Konow.

1.0; shape and posterior subbasal teeth of serrulae as in Fig. 8; maximum number of posterior subbasal teeth - 28 ----------4 First recurrent vein joins CUB in the first half; RCV gently curved (Fig. 2); postocellar area black in male; matetarsal joints not infuscated; ratio of height and width of supraantennaltubercles as 1 : 1.75; clypeus shallowly emarginate in male; posterior slope of scutellum longitudinally wrinkled; LID : IDMO = 3: 3.7; OOL : POL : OCL = 2.5: 1.- : 1.7; ratio of antennal segments 1-9 as 1.0: 0.5: 3.0: 2.5: 1.5: 1.25: 1.1: 1.0, ITD : ICD = 4.0: 1.0; shape and posterior subbasal teeth of serrulae as Fig. 6; maximum number of posterior subbasal teeth - 26 ----------*P. nefaensis* sp. nov.

4. Distal half of 5th, 6-9 antennal joints entirely black; propodeum and abdomen with all tergites reddish brown; wings yellowish hyaline, stigma and costa light reddish brown ---------- *P. privus* Konow, 1903. Base of antenna infuscated, only 4th and 5th joints partly fulvour; abdomen black; fulvous are : a paired spot on propodeum; a large spot on second tergite in middle and smaller middle spots on all tergites; wings smoky with faint purplish tinge, costa and stigma piceous --------*P.privus privus* ssp. Konow

(Nominal subsps) *P.privusvictoriaemontis* Malaise, 1945

Peus nefaensis sp.nov.

(Figs. 2,3,4,5,6)

Female : Length - 16 mm. Antennae reddish brown; extreme tip of segment 6th, 7-9 entirely black. Head reddish brown; yellow are : la-

brum and clypeus; supraclypeal area and inner orbits extending upto temples. Black are : apices of mandibles, broad stripe along base of clypeus, large frontal spot extending between antennae leaving supra-antennal tubercles, covering ocellii, reaching and extending along lateral furrows. Thorax black; anterolateral and posterodorsal angles of pronotum, tegula; large spot on mesonotal middle lobe, V shaped spot on lateral lobes; meso and metascutellii, scutellar appendage, postero-dorsal angle of mesepimeron; broad spot along covexity of mesosternum and metaepisternum; yellow. Abdomen reddish brown excepting nebulous spots on lateral deflexed sides of all abdominal tergites and distal, half of first sternite, -black. Legs reddish brown; black are : bases of pro-, meso; and meta coxae basal 1/3rd of all femora, leaving extreme proximal ends of all tibiae and extreme tip of metabasitarsus and claw joints. Wings yellowish hyaline, stigma and costa light reddish brown, venation dark brown to black.

Antenna slightly compressed in its distal half, 2.6 × head width; segment 1-9 in ratio 1 : 0.5 : 3.0 : 2.6 : 2.0 : 1.5 : 1.25 : 1.1 : 1.0 Clypeus truncate with smooth anterior margin. Labrum wider than long with subacuminate anterior margin. Malar space 1.5 × diameter of median ocellus. Ratio of height and width of supraantennaltubercles as 1 : 1.75. LID : IDMO = 3.0 : 3.7; OOL : POL : OCL = 2.5 : 1.0 : 1.7. Frontal area below the level of eyes. Supraantennal tubercles distinctly elevated, sloping posteriorly and abruptly cut off from frontal ridges. Circum, inter and postocellar furrows sharp. lateral furrows deep, continuous with lateral foveae and reaching hypothetical hind margin of head. Postocellar area convex slightly longer than broad with median longitudinal carina. Head narrowing behind eyes. Scutellum pyramidally raised with thorn like pointed apex, well above the level of mesonotal lobes. Appendage carinate ITD : ICD = 4.0 : 1. Metabasitarsus shorter than following 3 joints combined.

Head shining, minutely punctured, punctures more prominent on frontal area. Mesonotum densely and minutely punctured; mesoscutellum distinctly punctured along tangent lines from apex, its posterior slope longitudinally wrinkled. Appendage with few punctures, Mesepisternum rugose with irregular dense punctures. Basal two abdominal tergites polished, all other microstriated.

lancet as in Fig. 5 with 31 serrulae.
Maximum number of posterior subbasal teeth is 26 Fig. 6.
Male : Length - 14 mm. Similar to female except, post-ocellar area black; roughly triangular black spot on propodeum, all black markings on pro-, meso and metalegs reduced to a diminishing size; clypeus shallowly emarginate.

Penis valvè - Fig. 3.
Gonoforceps - Fig. 4.
Holotype : Female, Arunachal Pradesh, Bombila, 2600 meter, 19.5.1993.
Paratype : 1 Male with same data as of holotype.
Population variation : Single specimen of each sex examined.

Entomology : The name is based on the abbreviated form of region NEFA (North Eastern Frontier Area), from where the species is reported.

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Record of New Ascid Mites (Ascidae: Acari) Infesting Insects in Tamil Nadu, India

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Abstract: The paper records three Ascid mites, associated with insects in Tamil Nadu, of which two are new to science. The mites reported are *Blattisocius apisassociae* sp.nov. on *Apis cerana indica* (Apidae: Hymenoptera) *B. othreisae* sp.nov. on *Othreis sp* (Noctuidae: Lepidoptera) and *Lasioseius lindquisti* Nasr and Abou - Awad (1987) on pentatomid bug (Pentatomidae: Hemiptera).

Key words: *Blattisocius, Lasioseius*, Ascidae, Insects.

INTRODUCTION

The members of the family Ascidae are important due to their occurrence in all locations. Some species are fungivorous, pollen feeders, predators on young saptophytic mites, insects and nematodes (Moser 1975). Ascid mites are also phoretic on insects, myriapods and even humming birds (Lindquist and Evans, 1965). Some Ascid mites are aerial predators in ochards and prey on phytophagous mite. In addition some act as predators of mites in stored grain.

During screening and study of mites associated with insects in Tamil Nadu, two net species and another already known species of mites belonging to the family Ascidae were encountered which are described and illustrated.

All the measurements are given in microns. The types and paratypes are deposited in the Acarology collections of the Department of Agricultural Entomology, TNAU, Coimbatore - 641 003 India.

***Blattisocius apisassociae* sp.nov** (Figures 1 to 4)

Female: Length 493 and width 310; dorsum with 31 pairs of long smooth dorsal setae, jl anteriorly directed and widely placed. Dorsal shield entire; 437 long and 192 wide. Integument well striated and bearing long smooth setae.

Venter with three distinct body shields; the sternal shield 103 long and 70 wide bearing three pairs of sternal setae and a pair of metasternal setae away from the shield. Epygynial shield much longer (141 long) than wide (47) with a prominent inverted V shaped notch with a pair of setae at the posterior end Metapodal sclerite narrow and long; the ventrianal shield truncate, tapering posteriorly, 150 long and 70 wide; with 3 pairs of ventral setae; a pair of long and smooth anal setae which are long and smooth; adanal setae relatively smaller. The setae at the posterior end of the body is longer than other body setae, ventrum is well striated excepting the areas convered by ventral plates and platelets from where the ventral setae arise.

Gnathosoma less sclerotized, measuring 235 long, and 70 wide; chelecerae with dentate chela; palp tarsal apotele with a pair of thick tines, other characters typical to the genus.

Legs: coxae of legs I to IV with a small spur, each leg and in a pair of claws, pretarsus not so prominent in the Ist pair; legs I to IV measure 446, 329, 376 and 512 long respectively.

Leg chaetotaxy: Coxae: 2, 1, 1, 1: trochanter: 3, 3, 4, 2, femora: 8, 8, 6, 3; genua: 11, 8, 8, 10: tibia: 10, 10, 6, 10: tarsus: 25, 10, 10, 13.

Types: A holotype female marked on the slide along with another female, India; Tamil Nadu: Coimbatore; 1/xii/87, ex-*Apis cerana indica*

(Apidae: Hymenoptera) Coll: M. Mohanasundaram (No 46/2).

Diagnosis: This species resembles *Blattisocius tersalis* (Berlese, 1918) in general facies and chaetotaxy of venter, but differ in the body size, and in having less number of setae (31 pairs) on dorsal shield than *B.tarsalis* (34 pairs). In addition it lacks setae zl. The primary differentiating character is the presence of a deep notch at the posterior of epigynial shield.

Relationship to the host: The present form was isolated from the bees collected from lights. According to Baker and Yunker (1964) and Lindquist and Evans (1965) Ascid mites are aerial predators in orchards, where they prey on phytophagus mites. Moreover, they are also phoretic on humming birds, utilizing them for transportation from flower to flower where they feed on other arthropods or on pollen. Considering this, it is likely that this species is a pollen feeder or a predator on other mites and uses the bees as carrier from one plant to another. Thus the relationship might be a simple accidental phoresy in this case.

***Blattisocius othreisae* sp.nov.** (Figures 5-16)

Female: The dorsal shield 510 long, entire in both the sexes, idiosoma 692 long and 480 wide. Dorsum with 12 pairs of setae on the shield and five pairs on the soft integument. Mostly the dorsal setae being simple and short and 10 long; jl directed backwards and widely spaced. Posterior margin of the dorsal shield with a pair of long and stout setae 60 long. Dorsal shield smooth except with reticulate pattern anteriorly. The integumental region is well straited.

Venter: The sternal shield is not so distinct as wide as it's length (50) smooth with three pairs of long simple setae and lack any reticulation pattern. The first pair of sternal setae st1 is slightly off the shield in front; epigynial shield broad anteriorly and narrow medialy, with typically one pair of simple setae; The anal shield small, without pattern bearing a pair of simple paraanal and an equal sized anal seta; Opisthosoma prominently striated except the area covered by the shields and platelets; with two pairs of setae.

Gnathosoma 220 long and 90 wide, less sclerotized; with two pairs of long, slender and convergent corniculi, chelicerae with slender and long chela, fixed digit with seven strong teeth, palpal tarsi with forked tines.

Legs: The coxae of legs bears a stout spur. The femora and genua of leg I bears a pair of long smooth setae each; so also the tarsi - IV bear 3 pairs of long smooth stout setae. The empodium is four lobed. The legs I-IV measure 310, 260, 236 and 250 long, respectively. Leg chaetotaxy: Coxae: 2, 2, 2, 2: trochanter: 5, 5, 5, 4: Femora: 10, 8, 7, 3: Genua: 9, 8, 8, 5: Tibia: 9, 4, 6, 5: Tarsus: 28, 19, 15, 16.

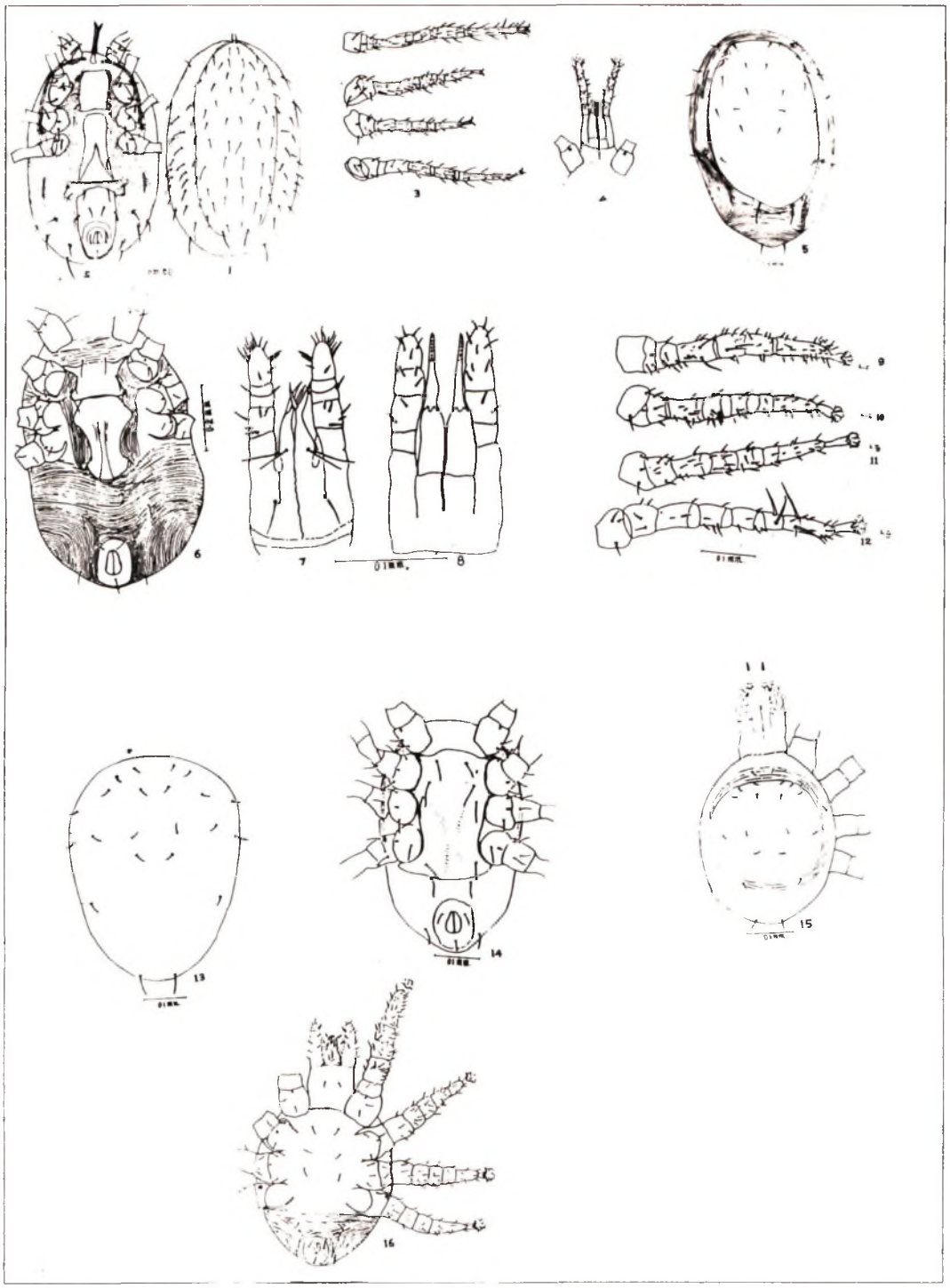
Male: The dorsal shield is entire, fully covering the dorsum, 500 long and 366 wide, without any distinct pattern, bearing 11 pairs of simple short setae recurved at an angle, each 10 long. The posterior end of dorsal shield with a pair of setae each 40 long.

Sterno-genital shield 288 long and 180 wide with faint patterns and four pairs of long smooth setae each 40 long arranged as figured. The anal shield 96 long and 80 wide with a pair of paranals and an equal sized anal setae; Integument on either side of the anal shield with a pair of long and smooth setae. Gnathosomal characters and leg characters and leg chaetotaxy similar to females. The legs I to IV: measure, 470, 420, 460 and 580 long respectively.

Nymph: The mite is flat and plumpy with enlarged podosoma occupying nearly one-third of the idiosoma. The body is almost oval, the idiosoma measures 370 long and 300 wide without any distinct pattern and plates both on the dorsum and venter dorsal shield is faint with 11 pairs of short, smooth, recurved setae 8 long, caudal setae 22 long.

Ventral shield not developed. Anal shield distinct three pairs of long smooth setae flanked by a pair of setae on either side situated on the integument. Opisthosomal venter striated.

Gnathosoma 180 long and 100 wide, quiet stumpy with long slender converging cornicles, individual palpal segments not distinct however



Figures 1 to 4: *Blattisocius apisassociae* sp. nov. 1. Dorsal view; 2. Ventral view; 3. Legs I to IV; 4. Gnathosoma; Figures 5 to 16 *Blattisocius othreisae* sp. nov. 5. Dorsal view of adult male; 6. Ventral view of adult female; 7. Gnathosoma, ventral view; 8. Gnathosoma, dorsal view; 9 to 12. Legs I to IV of adult female; 13. Dorsum of adult male; 14. Venter of adult male; 15. Dorsum of nymph; 16. Venter of nymph.

the palpal tarsi bear forked tines as in the adults.

Leg characters quiet different from those of adults: legs stumpy and flat with short setae; pretarsi distinct with a pair of claws and four lobed soft empodium; Leg I to IV measure 310, 260, 236 and 250 long respectively.

Leg chaetotaxy: Coxa: 2, 3, 3, 0: trochanter: 2, 4, 3, 1: femora: 11, 6, 4, 5 genua: 7, 5, 6, 4: tibia: 9, 6, 6, 4: tarsus: 20, 6, 11, 10.

Types: A holotype ♀ and an allotype ♂ marked on the slide, with several larvae: India: Tamil Nadu: Coimbatore: 25, VIII. 1987 ex *othreis* sp. (Noctuidae: Lepidoptera) fruit sucking moth, Coll.C. Chinniah (No.3) several paratype slides with collection data same as for the holotype.

Diagnosis: The present form resembles *Blattisocisus mali* (Oudemans 1929) in the general facies and adult characters, but differs, in having reduced number of setation of the dorsum, shape and arrangement of ventral shield in female that lacks pattern. One unique feature is the presence of a pair of long, stout and smooth setae on femora I and genua I and two pairs of macro setae on the tarsi IV; the chelae always have 7-8 teeth, in adults and only four teeth in nymphs.

Relationship to the host: The hyaline, soft bodies slightly sclerotized mites were isolated from the Noctuid-fruit sucking moth collected from the light trap during routine survey. The mites were found firmly attached to the inside of wing base and the sides of the abdomen (near wing joint) amidst the dense body scales and hairs, making the mites difficult to spot. Since all the stages such as nymphs male and female could be collected from a single moth it seems to be an ecto-parasite of the noctuid moth. According to Treat (1969) the mites of the genus *Blattisocius* are often found to occur on noctuid moth. *Blattisocius* has been recorded earlier on a noctuid *Epizewxis aemula* Hubner.

Lasioseius lindquisti Nasr and Abou - Awad (1987)

Materials studied: India: Tamil Nadu, Coimbatore 27.8.1987 ex-pentatomid bug (Pentatomidae - Hemiptera) Coll. C. Chinniah; deposited at Acarology laboratory TNAU - coimbatore - 3.

Relationship to the host: The light yellow coloured mites were isolated from an unidentified pentatomid bug collected with light trap (Pentatomidae: Hemiptera). All the mites were alive and found active all over the body. These mites did not possess any sort of phoretic modification. The ascid mites are effective predators on the eggs of beetles and bugs. Since all the mites collected were females, it seems they are phoretic on the bugs. This species is however new to Indian sub continent. It was originally described from Egypt by Nasr and Abou-Awad (1987).

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Biological Suppression of the White Spider Mite *Oligonychus iseilemae* (Hirst) on Coconut Foliage

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Abstract: *Oligonychus iseilemae* is one of the spider mite pests on the coconut palm foliage. This mite infests the ad-axial surface of the leaflets in colonies and the feeding results in the drying of the affected foliage. Natural predator complex of *O. iseilemae* comprised six species of spider. *A. (A.) paraaerialis, A. (T.) eucalypticus, Cunaxa setirostris* and *Agistemus* sp. are the major mite predators. Insect predators comprised Coccinellidae, Cecidomyiidae, Chrysopidae and Thripidae. Clubionid spiders are also dominant predators on this mite pest. The biology, predator-potential, seasonal occurrence and predator-prey ratio of the important predators were discussed.

Key words : Coconut, Spider mite, *Oligonychus iseilemae*, Mite predators, Phytoseiidae, Cunaxidae, Stigmaeidae, Predator-potential, Seasonal incidence

INTRODUCTION

Biological suppression of the spider mite pests of agricultural crops is possible by exploitation of the regulatory pressure exerted by the natural predators such as mites, insects and spiders. The phytophagous tetranychoid mites on the coconut palm comprised twelve species of spider mites and six species of false spider mites, infesting the foliage, inflorescence and nuts (Sathiamma, 1991). These mites usually occurred as occasional pests, but under favourable conditions their sporadic outbreaks could result in substantial crop losses.

Oligonychus iseilemae and *Tetranychus ludeni* are the two spider mite species infesting the coconut palm foliage (Sathiamma, 1985; 1988). The immature and adult stages of these mites sucked sap from the foliage. The affected parts get dried up. Sathiamma (1991, 1993) observed that weather parameters and the associated natural enemies governed the seasonal abundance of *O. iseilemae* under field conditions. Mite, insect and spider predators played an effective role in the natural biological suppression of these phytophagous species. Sathiamma (1992) discussed the role of the mite predators in the biological suppression of the mite pests of the coconut palm. Investigations were carried out in detail on the natural enemies of *O. iseilemae*, the prey consumption, biology, predator-prey ratio and seasonal incidence of the important predators and the results of these studies are discussed in this paper.

MATERIALS AND METHODS

Observations on the predators of *O. iseilemae* were recorded, every fortnight for two years (1985 and 1986), from two leaves each from ten sample coconut palms of the age group 3-4 years. The predators were counted on the basis of the actual observations on their feeding habits. A catalogue of the mite, insect and spider predators was prepared. The data were also used for studying the predator-prey ratio and seasonal intensity of the predators & prey in the field.

The prey consumption and the biology of the important predators were studied under laboratory conditions in Petri-plate cages. Fresh coconut leaflets were cut into bits (3.5 × 2.5 cm) and kept in petri-plate over water soaked cotton pads and the predators were released on these bits of leaflets for egg laying. *O. iseilemae* nymphs and adults were also released on these leaflets as prey for the predators. Data on the number of eggs laid and the period taken to complete the egg to adult stages were recorded daily at regular intervals (9.30 hrs and 16.30 hrs). Observations were recorded in the laboratory at a mean temperature 28 ± 1°C and relative humidity of 68 ± 2%. The predators as

Table 1. Natural predator complex of *Oligonychus iseilemae* (Hirst) on coconut palm foliage

Class/Subclass	Order	Family	Name of predator
Acarina	Mesostigmata	Ascidae Phytoseiidae	*Lasioseius sp.* *Amblyseius (Amblyseius) paraaerialis* Muma *A. (Typhlodromalus) eucalypticus* Gupta
	Prostigmata	Cheyletidae Cunaxidae Eupodidae Stigmaeidae	Unidentified *Cunaxa setrostris* (Hermann) *Eupodes sp.* *Agistemus sp.*
Arachnida	Araneae	Clubionidae	*Cheiracanthium sp.*
Insecta	Coleoptera Diptera	Coccinellidae Cecidomyiidae	*Stethorus keralicus* Kapur Unidentified

soon as they hatch from the egg were provided with 30-50 numbers of the prey containing the egg, larva nymph and adult stages. The number of prey and the stage of the prey consumed at each stage of the predator was separately recorded at 24 h interval. Mean prey consumption was worked out from the data collected. Separate cages were maintained for the different species of predator under this experiment.

Results and Discussion

Natural predator complex of the coconut white spider mite *O. iseilemae* comprised six species of mites, two insects and one spider. They belonged to nine genera (including two unidentified ones) of nine families and five Orders (Table 1). These predators occupied the same habitat and co-existed with the colonies of prey mites or remained scattered on the mite infested coconut foliage.

Species of *Amblyseius* (Phytoseiidae) were the major predators of *O. iseilemae*. There were two species, *Amblyseius (Amblyseius) paraaerialis* Muma and *A. (Typhlodromalus) eucalypticus* Gupta. *Cunaxa setirostris* (Cunaxidae), *Agistemus* sp. (Stigmaeidae), *Lasioseius* sp. (Ascidae) and an unidentified species of Cheyletidae were the other mite predators recorded. These predators are new records on the prey and the host palm.

A. (A.) paraaerialis

Adults and immature stages of *A. (A.) paraaerialis* fed on the eggs and motile stages of the prey. These dominant predators occurred in abundance during the peak period of incidence of *O. iseilemae*. The larvae and protonymphal stages of the predator consumed the immature stages of the prey; the deutonymphal predator fed on the immature and adult stages of the prey and the adult predator preferred the eggs rather than the motile stages of the prey.

The prey consumption per larva was 2.8 ± 0.2, protonymph 3.1 ± 0.4, deutonymph 3.4 ± 0.3 and adult female 5.4 ± 0.9 prey during its developmental period. As compared to the females, the adult male predator consumed less, the average feeding was 1.3 ± 0.2, 2.0 ± 0.3, 2.3 ± 0.2 and 2.7 ± 0.3, respectively, for larvae, protonymph, deutonymph and adult stages (Table 2).

The female predator laid 15-35 eggs during its life period of 10-20 days. It completed the egg to adult period in 4.5 ± 0.1 days, with an egg period of 1.2 ± 0.1, larval 1.0 ± 0.0, protonymphal 1.2 ± 0.1 and deutonymphal 1.1 ± 0.1 days. Whereas, the male predator took only 4.2 ± 0.1 days to complete the development from egg to adult stage (Table 3). *A. (A.) paraaerialis* is also a predator on the coconut

Table 2. Prey consumption by *Amblyseius (A.) paraaerialis*, *A. (T.) eucalypticus* and *Cunaxa setirostris* predacious on *Oligonychus iseilemae* in the laboratory*

Predator stage	Prey consumed			
	A. (A.) paraaerialis		*A. (T.) eucalypticus*	*C. setirostris*
	Male	Female	Female	Female
Larva	1.3 ± 0.2	2.8 ± 0.2	2.8 ± 0.3	8.3 ± 1.1
Protonymph	2.0 ± 0.3	3.1 ± 0.4	3.7 ± 0.7	11.8 ± 1.4 **
Deutonymph	2.3 ± 0.2	3.4 ± 0.3	5.8 ± 0.6	
Adult	2.7 ± 0.3	5.4 ± 0.9	9.0 ± 0.5	14.9 ± 1.7

* Mean of 10 observations

** Protonymph and deutonymph stages combined

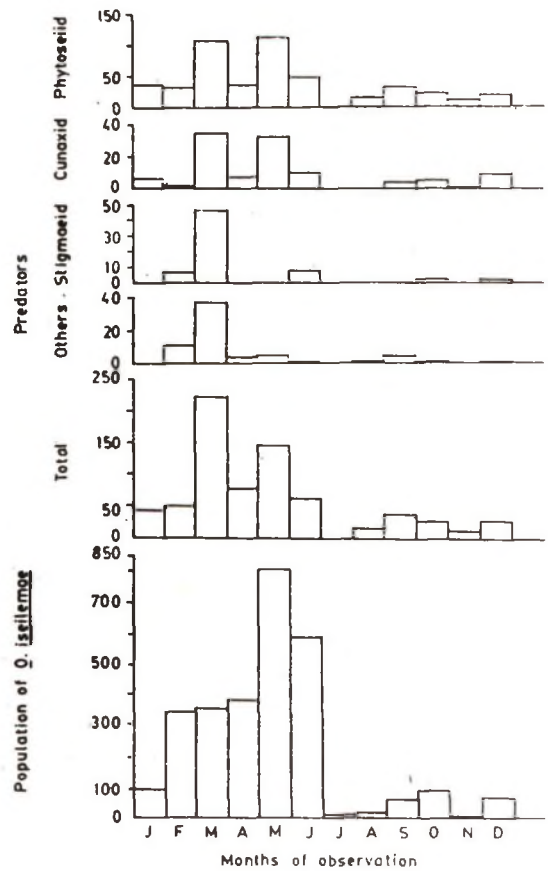

Fig 1. Fluctuations in population density of Oligonychus iseilemae and the associated predators (Mean of 1985 and 1986)

Table 3. Duration of Life stages (Mean ± SE) of *Amblyseius (A.) paraaerialis*, *A. (T.) eucalypticus* and *Cunaxa setirostris* in the laboratory *

Life stage	Duration (days)			
	A. (A.) paraaerialis		*A. (T.) eucalypticus*	*C. setirostris*
	Male	Female	Female	Female
Egg	1.0 ± 0.0	1.2 ± 0.1	1.0 ± 0.0	2.1 ± 0.2
Larva	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.9 ± 0.1
Protonymph	1.0 ± 0.0	1.2 ± 0.1	1.2 ± 0.1	2.4 ± 0.1 **
Deutonymph	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	

* Mean of 10 observations

** Protonymph and deutonymph stages combined

red spider mite *T. ludeni* and the rate of consumption was 1-4 prey per day. This predator also fed on *A. eucalypticus*, another phytoseiid predator on *O. iseilemae*.

A. (T.) eucalypticus

A. (T.) eucalypticus inhabits the coconut leaflets amidst colonies of *O. iseilemae*. They fed on all stages of the prey. The predator larvae consumed 2.8 ± 0.3, protonymph 3.7 ± 0.7 and deutonymph 5.8 ± 0.6 prey per predator during its life period. Adult female predator consumed 9.0 ± 0.5 and the male 2.5 ± 0.2 prey per day (Table 2). But, the predator larva consumed only the egg and larval stages of the prey and the protonymph consumed the immature prey stages and the deutonymph and the adult predator on all stages of the prey.

The adult female laid only 5-8 eggs and completed the egg to adult period in 4.3 ± 0.2 days which comprised an egg and larval period of one day each, protonymphal 1.2 ± 0.1 and deutonymphal 1.1 ± 0.1 days (Table 3).

Cunaxa setirostris

The cunaxid mite *C. setirostris* is a very active and efficient predator on all the motile stages of *O. iseilemae*. The predator larva preferred the larval prey; the nymphal predator preferred the larvae and early nymphs of the prey and the adult preferred the prey nymphs and adults. Total prey consumption during its larval stage was 8.8 ± 1.1 and nymphal 11.8 ± 1.4 prey (Table 2).

The female predator lived on an average for 15.8 ± 0.7 days and laid 6.3 ± 0.7 eggs. The egg to adult period was completed in 6.3 ± 0.2 days. The egg period comprised 2.1 ± 0.2, larval 1.9 ± 0.1 and nymphal (inclusive of protonymphal and deutonymphal stages) 2.4 ± 0.1 days (Table 3).

Agistemus sp.

Species of *Agistemus* occurred at a low level in the field and it fed on all developmental stages of *O. iseilemae*. The predator larva fed on the larval prey, nymph fed on all immature stages of the prey and the adult fed on all life stages. The prey consumption ranged from 20.0 to 20.5 by larva, 3.7 to 30.0 by nymph and 3.3 to 31.0 by the adult.

Predator- prey ratio

Phytoseiidae and Cunaxidae are the dominant predators of *O. iseilemae*. In the field, the overall predator-prey ratio was 1 : 4.7, which varied from 1 : 0.5 during Novmber to 1 : 11.2 during June. High population of the prey occurred during March, when the predator-prey ratio was 1 : 2.6 and during May, the peak period of the prey, the ratio was 1 : 5.7.

Considering the phytoseiid predators, the predator-prey ratio varied from 1 : 0.5 to 1 : 13.6, with a peak population ratio of 1 : 7.4, during May. As compared to the Phytoseiidae, the cunaxid predators were comparatively low, with a peak during March, the predator-prey ratio being 1 : 10.7.

Seasonal abundance

The predators occurred in the field during all months of the year, except July (Fig. 1).

Table 4. Seasonal occurrence and mean population of the predators of *Oligonychus iseilemae* (Hirst) during the years 1985 and 1986 (Total palms observed - 10)

Month	Mites				Insects		Spiders
	Phytoseiidae	Cunaxidae	Stigmaeidae	Cheyletidae	Coccinellidae	Cecidomyiidae	Clubionidae
January	70	11	0	1	2	2	40
February	64	23	14	1	3	6	17
March	208	66	92	1	4	0	20
April	136	11	0	0	5	0	6
May	218	63	0	1	4	2	13
June	86	18	14	1	0	5	5
July	0	0	0	0	0	0	0
August	32	0	0	0	1	0	23
September	55	6	4	1	4	0	21
October	40	9	2	0	2	0	22
November	19	1	0	0	0	1	18
December	36	15	2	0	0	2	25

High population was recorded from January to May and very low to zero from June to December. Maximum predator population occurred from March to May. The seasonal occurrence of the predators followed a similar pattern as that of the prey. With increase in the population of *O. iseilemae* from January, the predator population gradually built up and reached the peak level in March. But, with the decrease in prey population the number of predators also became less and reached either a very low level or were totally absent. Predators influenced a decrease in the spider mite population and their beneficial role was observed when the population reached severe proportions.

The phytoseiid predators *A. (A.) paraaerialis* and *A. (T.) eucalypticus* were abundantly present as compared to the cunaxid, stigmae and cheyletid predators. High population of these mites occurred from March and May. A similar trend was noted in the cunaxid mites. Stigmaeid predators occurred in abundance during March. Population of cheyletid mites was extremely low and was present during January, March, May, June and September. Among the insect predators, Coccinellidae and Cecidomyiidae were the dominant ones. Clubionid spiders, particularly species of *Cheiracanthium* occurred throughout the year, except July (Table 4).

Increase in spider mite population on coconut was regulated by a number of factors including climate and natural enemies (Sathiamma, 1993). The present observations also revealed that the predators were capable of multiplying in large numbers and maintaining the spider mite population at a low level. This method of biological suppression of pests was successfully employed on a number of agricultural crops. According to Helle and Sabelis (1985), Huffaker *et al.*, (1970), Mc Murtry (1982) and Pickett and Gilstrap (1986) phytoseiid mites play an important role in the biological suppression of mite pests. *A. (A.) paraaerialis* and *A. (T.) eucalypticus* (Phytoseiidae) were the most dominant predators checking the population or *O. iseilemae* and *T. ludeni* in the field. These active predators occurred in abundance corresponding to the increase in prey mite population. They have a short life cycle, a high potential for multiplication and consumed all stages of the prey.

A. (Euseius) alstoniae (Gupta and Gupta, 1978), *A. (A.) nuciferae* (Gupta, 1986), *A. largoensis* (Schicha and Gutierrez, 1985) and an unidentified species of phytoseiid (Cayme

and Gapasin, 1987) were the other species of phytoseiid mites recorded on coconut. The economic importance of these species is not known, but for *A. (E.) alstoniae* feeding on *Raoiella indica,* the false spider mite on coconut.

C. setirostris is one of the potential predators of *O. iseilemae*. It is world wide in distribution and known to be an efficient predator of tetranychid mites. It has all the essential prerequisites of an efficient predator with a good searching ability, shorter life span, prolonged longevity and high rate of prey consumption.

Species of *Agistemus* are potential predators of tetranychoid mites (Gupta, 1985; Gupta *et al.*, 1971). The present observations clearly indicated that combined with the phytoseiid and cunaxid predators, *Agistemus* sp. effected successful natural suppression of the spider mite population during the period of mite abundance.

The insects, particularly Coccinellidae are well known predators on spider mite pests. *Stethorus fijiensis, S. expectatus* and *S. exsultabilis* on coconut spider mites (Cayme and Gapasin, 1987; Chazeau, 1983) and *S. Keralicus* on the arecanut false spider mite (Daniel, 1979) are some of the recorded predators.

The spiders are quite abundant in the coconut ecosystem. *Cheiracanthium* sp. is one of the most efficient predators of *Opisina arenosella,* the leaf eating caterpillar pest of coconut (Sathiamma *et al.,* 1987) and this species consumed the spider mites to the coconut foliage.

Along with the mite predators, the insect and spider predators effected significant suppression of the spider mite population on coconut foliage. Some of these predators are individually insignificant, but collectively their abundance coincided with prey abundance and effected biological suppression of the tetranychid mite *O. iseilemae* infesting coconut palm.

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Host Plant-Induced Response to Insecticides and Haemolymph Esterase Patterns in *Spodoptera litura* (Fabricius)

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Abstract: Third instar larvae of *Spodoptera litura* (Fab.) fed on cotton and tomato were most susceptible followed by those fed on groundnut, tobacco and castor to all the insecticides tested viz., endosulfan, monocrotophos, chlorpyriphos, carbaryl, cypermethrin and fenvalerate. Haemolymph esterase patterns revealed that the larvae fed on cotton and tomato recorded four and five esterase bands respectively while the larvae fed on tobacco recorded eleven bands. Susceptibility to insecticides decreased with the increase in the number of esterases which might have contributed to the detoxification of the insecticides.

Key words: *Spodoptera litura*, insecticide-susceptibility, host plants, haemolymph esterases, electrophoresis

INTRODUCTION

Tobacco caterpillar, *Spodoptera litura* (Fab.) has been posing a serious threat to several agricultural and horticultural crops during *rabi* in garden land and dryland ecosystems in Andhra Pradesh. Frequent failures of insecticides to contain the pest on various crops are due to development of resistance (Reddy and Prasad 1991). Food of insects largely determines their metabolic system which inturn decides its response to insecticdes. Hence information on the response of the insect feeding on different food plants to insecticides and the associated biochemical evidence is vital in ecotoxicological approach.

Differential response to insecticides in *S. litura* with reference to host plants was earlier documented by Rattan Lal and Nayak (1963). Biochemical evidence for such differential response was also provided by Salama *et al.*, (1992). In this context, an attempt was made to study the insecticide-susceptibility of *S. litura*, fed on five common host plants viz., castor, tobacco, groundnut, tomato and cotton.

MATERIALS AND METHODS

Bioassay

Larvae of *S. litura* were reared in rearing troughs (25 × 20cm) using the leaves of each host plant viz., castor (*Ricinus communis* L.), tobacco (*Nicotiana tabacum* L.), groundnut (*Arachis hypogaea* L.), tomato (*Lycopersicon esculentum* Mill.) and cotton (*Gossypium hirsutum* L.). Newly moulted third instar larvae of second generation were bioassayed against endosulfan, monocrotophos, chlorpyriphos, carbaryl, cypermethrin and fenvalerate by topical application of 15 µl of insecticidal solution on dorsum of prothorax. The dose-mortality data recorded 24 hours after treatment were subjected to probit analysis (Finney, 1952).

Electrophoresis

Haemolymph samples of third instar larvae fed on respective host plants were collected by puncturing proleg and drawing the exuded haemolymph into a glass tube to which few crystals of 1-phenyl-2-thiourea were added to prevent melanization. The haemolymph sample was centrifuged at 10000 × g for 30 minutes and 10 per cent sucrose solution was added.

Polyacrylamide gel electrophoresis (PAGE) was performed in an electrophoresis units (Mc. Dalal & Company, Madras) on a 1.5 mm vertical non-denaturing slab gel for 4 hours at a constant current of 30 mA until the tracking dye (Bromophenol blue) migrated to the end of

Table 1. Probit analysis of dose-morality response in third instar larvae of *S. litura* fed on different host plants of different insecticides.

Insecticide	Host Plant	Chi-square df=3	Slope 'b'	LC_{50}	Fiducial limits 95%	
Endosulfan	Castor	0.0728	1.2509	0.0679	0.0384	0.1816
	Tobacco	0.4693	1.2817	0.0606	0.0323	0.0981
	Groundnut	0.0193	0.8993	0.0394	0.0067	0.0758
	Tomato	0.0188	0.8981	0.0098	0.0016	0.0190
	Cotton	0.0472	0.3218	0.0072	0.0008	0.0139
Monocrotophos	Castor	0.0461	0.9000	0.0576	0.0071	0.1107
	Tobacco	0.5714	0.9643	0.0451	0.0218	0.1047
	Groundnut	0.0104	1.2036	0.0332	0.0148	0.0632
	Tomato	0.1323	1.0222	0.0054	0.0007	0.0084
	Cotton	0.3508	1.1480	0.0043	0.0016	0.0113
Chlorpyriphos	Castor	0.0816	0.9700	0.0308	0.0118	0.0600
	Tobacco	0.0193	0.8993	0.0197	0.0034	0.0379
	Groundnut	0.2382	1.3071	0.0255	0.0106	0.0442
	Tomato	0.0188	0.8981	0.0098	0.0016	0.0190
	Cotton	0.0330	0.7194	0.0013	0.0001	0.0172
Carbaryl	Castor	0.0592	1.2372	0.1349	0.0742	0.4036
	Tabacco	0.0182	0.8994	0.0786	0.0139	0.1526
	Groundnut	0.0400	0.9293	0.0386	0.0075	0.0727
	Tomato	0.4855	0.7542	0.0052	0.00001	0.0123
	Cotton	0.0461	0.9459	0.0144	0.0018	0.0278
Cypermethrin	Castor	0.0119	1.2000	0.0331	0.0146	0.0630
	Tobacco	0.0318	1.0118	0.0321	0.0064	0.0586
	Groundnut	0.0182	0.9004	0.0197	0.0034	0.0380
	Tomato	0.0470	0.9456	0.0072	0.0009	0.0139
	Cotton	0.1312	1.0231	0.0055	0.0005	0.0106
Fenvalerate	Castor	0.0183	0.8984	0.0078	0.0013	0.0151
	Tabacco	0.0182	0.8993	0.0039	0.0007	0.0076
	Groundnut	0.0134	1.1999	0.0066	0.0029	0.0126
	Tomato	0.0485	1.0375	0.0017	0.0004	0.0029
	Cotton	0.3488	1.1499	0.0009	0.0003	0.0023

the gel. The running gel was prepared with 1.5 M Tris-HCl buffer (pH 8.9) and the stacking gel (2.6%) with 0.5 M Tris-HCl buffer (pH 6.7). Both electrode chambers were filled with Tris-Glycine buffer (pH 8.3).

After electrophoresis the gel was soaked in 0.5 M borate buffer (pH 4.1) for 90 minutes at 4°C. The gel then was rinsed rapidly in two changes of double distilled water. The gel was stained for esterolytic activity by incubating the gel at 25°C in a solution containing 100 mg of α- naphthyl acetate as a substrate, 100 mg of fast blue RR salt as diazocoupler and 200 ml of 0.1 M phosphate buffer (pH 6.5). After incubation the gel was destained in 7 per cent acetic acid. The zymogram was prepared and quantified by calculating the relative mobility of isozyme bands.

$$\text{Relative mobility (Rm)} = \frac{\text{Distance travelled (cm) by enzyme band}}{\text{Distance travelled (cm) by dye front}}$$

RESULTS

Susceptibility of *S. litura* fed on different host plants to insecticides:

Estimates of probit analysis of dose-mortality response in third instar larvae of *S. litura* fed on different host plants to certain insecticides are presented in table 1. Chi-square values in all the bioassays were found non-significant at 5 per cent level indicating the

Table 2. Relative mobility (Rm) of haemolymph esterases of third instar larvae of *S. litura* fed on different host plants and their intensity

Sl. No.	Rm	Group*	Number of bands and their intensities				
			Castor	Tobacco	Groundnut	Tomato	Cotton
1.	0.06	I	L (1)	--	L (1)	L (0.5)	L (0.5)
2.	0.11	I	L (1)	--	L (0.5)	--	--
3.	0.14	I	L (1)	--	L (0.5)	L (0.5)	L (0.5)
4.	0.37	II	L (1)	L (1)	L (1)	--	--
5.	0.38	II	--	M (1.5)	L (1)	--	--
6.	0.41	II	L (1)	--	--	--	--
7.	0.42	II	--	M (2)	M (1.5)	--	--
8.	0.46	II	L (2)	--	L (1)	L (0.5)	--
9.	0.48	II	L (1)	L (1)	L (1)	L (0.5)	L (0.5)
10.	0.53	II	D (2)	L (1)	M (1.5)	M (1.5)	M(1.5)
11.	0.54	II	M (1)	--	M (1.5)	--	--
12.	0.62	II	L (1)	M (1)	--	--	--
13.	0.63	II	--	L (1)	--	--	--
14.	0.66	II	--	L (1)	--	--	--
15.	0.75	III	--	M (1.5)	--	--	--
16.	0.79	III	--	M (1.5)	--	--	--
17.	0.83	III	--	L (1)	--	--	--

* Range of Rm values in Group I = 0.00 to 0.24, Group II = 0.35 to 0.64, Group III = 0.75 to 0.89
Figures in parenthesis indicate thickness of bands (mm)

homogeneity of test population and a close fit between observed and expected responses.

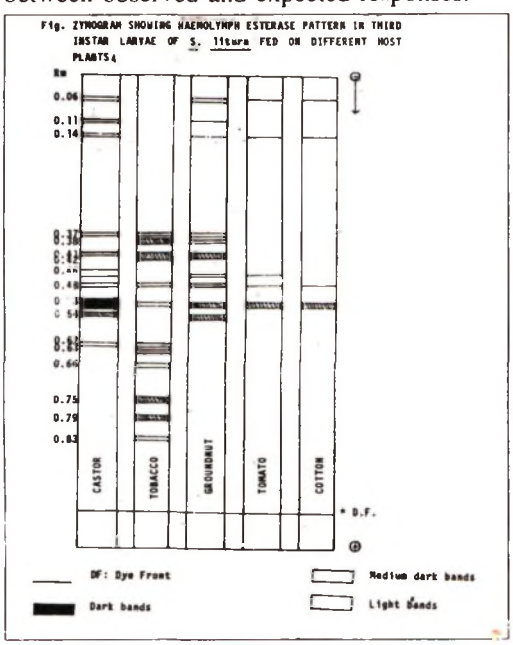

Fig. ZYMOGRAM SHOWING HAEMOLYMPH ESTERASE PATTERN IN THIRD INSTAR LARVAE OF S. litura FED ON DIFFERENT HOST PLANTS

Comparison of LC_{50} values of each insecticide against the larvae fed on different host plants revealed that the larvae fed on cotton and tomato were the most susceptible to the insecticides tested followed by those fed on groundnut, tobacco and castor. Dose-mortality response was markedly higher in the larvae fed on castor to insecticides, particularly carbaryl, endosulfan and monocrotophos. Higher slope coupled with higher tolerance to insecticides was recorded in the larvae on castor.

Haemolymph esterase patterns

Hemolymph esterase patterns investigated through electrophoresis revealed that least number of esterase bands were present in larvae fed on cotton (four) and tomato (five) while higher number of hands with higher intensity were found in the larvae fed on tobacco (eleven), castor (ten) and groundnut (ten) Table 2, Fig. 1). The esterases of low molecular weight under group III were found only in tobacco-fed larvae.

Findings on insecticide-susceptibility and the haemolymph esterase patterns suggested that susceptibility to insecticides decreased with increase of haemolymph esterases in the test larvae which were in turn influenced by the quality of food.

DISCUSSION

The larvae fed on cotton and tomato were the most susceptible to all the insecticides tested followed by those fed on groundnut, tobacco and castor. Rattan Lal and Nayak (1963) reported that susceptibility to DDT, endrin and aldrin in *S. litura* was least on castor followed by cabbage and tobacco. According to Loganathan and Gopalan (1985) insecticide susceptibility in *H. armigera* was high on tomato. Refai *et al.*, (1979) found that tomato-fed *H. armigera* was very sensitive to insecticides and this was attributed to nutritional inadequacy of tomato due to higher water content and low content of proteins, fats and carbohydrates. More number of esterase bands on tobacco and castor might have contributed to higher tolerance to insecticides in the pest species. Terriere (1984) opined that the quantitative and qualitative nutritional factors influenced the ability of insect to synthesize and maintain its detoxification enzymes. Larvae fed on castor were highly responsive to carbaryl, endosulfan and monocrotophos. Higher slope coupled with higher tolerance to insecticides was recorded in the larvae on castor.

present findings throw light on the need for crop-based chemical control strategies in IPM of *S. litura* suggesting the possibility of dietary manipulations.

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Life Cycle and Sexual Dimorphism in Pupa of *Scatogranna submarginalis* Walk. (Lepidoptera: Noctuidae)

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Abstract : *Scatogranna submarginalis* Wlk. is one of the major pests of sunflower in western Uttar Pradesh. The insect completed its life cycle egg to adult in 30-50 days with an incubation period of 4 days under laboratory conditions at an average maximum 31.11°C±0.26°C and minimum 26.19°C ±0.38°C temperature and 40.88±0.88% relative humidity during March-April. The neonate caterpillars moulted five times and complete its caterpillar stage in six instars. The total larval period recorded was 14.50 days. The pupal period was 6.00±0.15 days with an adult longevity of similar duration. A distinct sexual dimorphism was observed at pupal stage.

Key words: Life Cycle, dimorphism, SEM, *Scatogranna submarginalist*

INTRODUCTION

Sunflower and castor being oil yielding crops of great economic importance are attacked by the caterpillars of *S. submarginalis* (Singh, 1988). Presently the insect has assumed the status of a regular pest of sunflower causing defoliation in western Uttar Pradesh. Several workers have described the life cycle of a number of noctuid species from Hadeninae namely Breeland (1958), Yadav (1972) and Kumar and Goel (1985). However, the life cycle of *S. submarginalis* is not reported. Considering the importance of *S. submarginalis* to the oil yielding crops, the present study on the life cycle and sexual dimorphism of pupa was undertaken.

MATERIALS AND METHODS

For studying the biology of *S. submarginalis*, the laboratory culture was developed on sunflower leaves from field collected caterpillars during March to April. A regular record of number of eggs, hatchability, larval instars, pre-pupa and pupal period, adult longevity and mortality were made at an average maximum 31.11°C±0.26°C and minimum 26.19°C±0.38°C temperature and 40.88% relative humidity. To study the eggs, different instars and pupae were preserved in K. A. A. D. for morphometrics. The developmental stages were measured with the help of ocular and stage micrometer combination. The exuvia of pupa were used for scanning photographs.

RESULTS AND DISCUSSION

Eggs: A single female laid 109 eggs in captivity in clusters, covering with anal tufts for two days during night on sunflower leaves. Each egg was spherical, brown, finally turns slaty gray in colour before hatching and measured 0.54±0.01 mm in diameter (Table 1). The incubation period remained for 4 days, and hatching percentage was recorded 89.45%. The egg chorion was eaten away by the larva at micropylar region making a hole for emergence.

Caterpillars: The newly emerged dark brown caterpillars congregated near the egg mass and started feeding on empty egg shells. The feeding then transferred to the schlerenchymatous part of tender leaves in between the veins. The

Table 1. Life period of *Scatogranna submarginalis* Wlk. during March to April

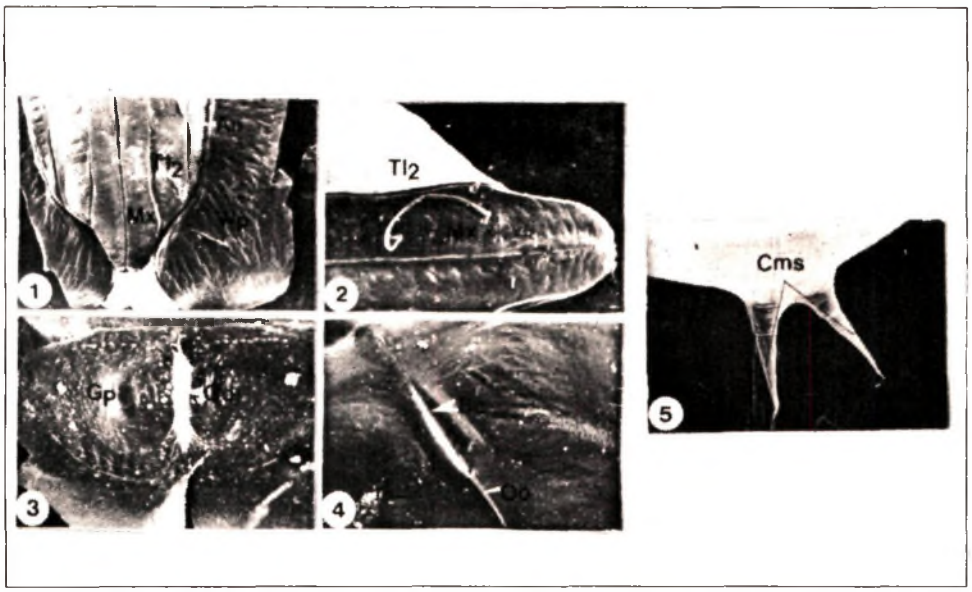
	Eggs	Ist instar	2nd instar	3rd instar	4th instar	5th instar	6th instar	Pre pupa	Pupa
Duration (Days)	4.00	2.00	2.00	1.20	2.00	2.40	3.10	1.80	6.00
		± 0.00	± 0.00	± 0.13	± 0.00	± 0.25	± 0.25	± 0.13	± 0.15
Length (mm)	0.54	1.30	4.40	7.65	10.10	18.65	32.70	21.60	16.50
	± 0.01	± 0.02	± 0.15	± 0.19	± 0.18	± 0.59	± 0.47	± 0.45	± 0.33
Width (mm)	0.54	0.25	0.71	1.19	1.51	3.15	4.70	3.60	4.50
	± 0.01	± 0.01	± 0.01	± 0.02	± 0.02	± 0.14	± 0.08	± 0.14	± 0.10
Mortality (%)	10.55	17.69	14.28	8.33	4.54	9.52	--	--	16.66
Min. Temp (°C)	26.17	23.50	25.50	26.50	26.50	26.00	26.62	27.60	27.39
	± 0.31	± 0.35	± 0.35	± 0.35	± 0.35	± 0.18	± 0.41	± 0.52	± 0.60
Max. Temp (°C)	30.17	28.50	30.00	31.50	31.50	31.50	31.87	32.30	32.67
	± 0.09	± 0.35	± 0.00	± 0.35	± 0.35	± 0.18	± 0.37	± 0.39	± 0.26
Rel. Humidity (%)	46.33 ± 03.31	55.50 ± 0.33	36.00 ± 0.00	40.00 ± 0.00	38.00 ± 01.49	34.50 ± 02.59	41.00 ± 01.77	39.25 ± 02.40	36.33 ± 01.83

Maximum Average Temperature	Minimum Average Temperature	Average Relative Humidity
31.11°C ± 0.260°C	26.19°C ± 0.38°C	40.88% ± 0.88%

Adult longevity = 6.00 days; Total life period = 30.50 days.

first instar larva measured 1.30±0.02 mm in length and 0.25±0.01 mm in width whereas the mature sixth instar caterpillar measured 32.70±0.47 mm in length and 4.70±0.08 mm in width. The second instar larva is green in colour having two spots, one on each lateral side of metathorax, which gets replaced by a black band developed at third instar stage. In the successive stages from second instar onwards, the caterpillar turns to dull brown in colour. The maximum mortality was recorded during Ist instar (17.69%) and minimum during IVth instar (4.54%) (Table 1). The last instar congregated between the leaves and formed a cocoon as to enter into a quiescent stage to convert into the pre-pupa. the size of the caterpillar was reduced to 21.60±0.45 mm in length and 3.60±0.14 mm in width before pupation. The prepupal period remained for 1.80±0.13 days (Table 1).

Pupa: The labial palpi proximally are bifid caudad to labrum whereas enter basally and reached upto 1/5 of the maxilla. Maxillary palp reaches more than 1/4 of the maxilla. The wing pads (Wp) covers the pupa upto the spiracle of Ab IV segment, meta-thoracic wing pad upto the posterior margin of Ab III leg lies adjacent to the maxilla in the form of a triangular sclerite and more than half of the maxilla, whereas the mesothoracic leg ($T1_2$) is slightly shorter than the maxilla (Mx) in both the sexes (Figs. 1 & 2). The antennae (An) are smaller than the mesothoracic leg in both the sexes. The ten segmented abdomen has three segments Ab VIII to Ab X with no power of independent motion. The male genital opening (Go) is cylindrically elongated and situated on the ventromeson of Ab IX in between the two rectangular elevated genital pads (Gp) (Fig. 3). In the female, the genital opening is associated apparently with Ab VIII and Ab IX. The genital opening of the female consists of the two confluent openings. The anterior bursa copulatrix (Bc) and the posterior ovipositional opening (Oo) (Fig. 4). similar to *S. marginalis*, the two separate openings in female pupae were also discussed in noctuids pupae viz. *Plusia orchalcea, Chalciope hyppassia* and *Agrotis biconica* by Kumar and Goel (1988), Singh and Goel, (1987) and Singh *et al.*, (1991) respectively. The intersegmental line



Figures 1-5. Scanning electron photomicrographs of pupa *Scatogranna Submarginalis* Wlk.
Fig. 1. Ventral view of pupa showing antennal elevation (An), *maxilla* (Mx) wing pads (WP) and second thoracic leg (Tl_2). x 40; 2. Ventral view of pupa showing male maxilla (Mx) and second thoracic leg (Tl_2). x 80; 3. Ventral view of male pupa showing two rectangular elevated gential pads (Gp) and a gental opening (Go) x 80; 4. Ventral view of female pupa showing bursa copulatrix (Bc) and ovipositional opening (Oo) x 160; 5. Posteroventral view of pupa showing creastral spines (cms) x 40.

between Ab VIII, Ab IX and Ab X is strongly curved towards the genital opening in the female. A slit like anal opening is situated on Ab X, and surrounded on each side by several prominent wrinkles in both the sexes. A puncture on each side of anal opening in both the sexes exists midventrally on Ab X. The cremaster has a pair of long pointed cremasteral spines (Cms) (Fig.5). There is no cremasteral setae on the cremaster.

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Influence of Male Age on Mating Capacity, Fecundity and Fertility of Mated Female Silkmoth, *Bombyx mori* L. Under High Temperature and High Humidity Conditions

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Abstract: The male silkmoths, *Bombyx mori L.* were mated at the age of 0, 1, 2, 3, 4, 5, 6 and 7 day old with 0 day old virgin females under high temperature and high humidity conditions during rainy season. The mating capacity of the males decreased significantly with the increase of age. A significant negative correlation (r= -0.907) was observed between age of males and mating capacity, whereas the total number of eggs laid by the mated females and its fertility did not differ significantly irrespective of her mating partner's age.

Key words: Mating, Oviposition, Fertility, *Bombyx mori*

INTRODUCTION

In silkmoth, the stimulus for oviposition and the influence of mating duration on fecundity and fertility have been reported by a number of investigators (Omura, 1939; Yamaoka and Hirao, 1973; Punitham *et al.*, 1987; Fugo and Arisawa, 1992). The age of the female moth influences the fertility level of the eggs (Paul *et al.*, 1992). *Bombyx mori* being an economically important insect, its population size has a direct bearing on the cocoon yield.

The usual practice is either to allow mating after emergence or to store the freshly emerged male moths at a lower temperature of 7°C for about a week. These males are preferentially used for mating with the virgin females depending on their availability. Under such recommended procedure the reproductive potential of the males is not ordinarily impaired (Krishnaswami *et al.*, 1973). Uptil now no information is available about mating capacity of male moth with the increase of its age up to 7 days and its reproductive potential under high temperature (27-32°C) and high humidity (76 - 92%) during rainy season. We conducted experiments to study the effect of male age on mating capacity and subsequent reproduction by female for production of viable eggs under adverse environmental condition.

MATERIALS AND METHODS

Multivoltine Nistari layings were brushed continuously for 7 days (7 lots) and reared under room conditions at temperature 27- 32°C and 76- 92% humidity in order to get 0 day old female moths (fresh females for 7 days). After spinning, the cocoons were harvested on the 5th day and the pupae were sexed. Freshly emerged males of first day were selected and 8 groups of males each were made. Simultaneously reserve batch for each group was also maintained separately for replacement with the same age male in case of mortality if any during the life span of adult. The males in each group were kept individually within a plastic cellule to avoid selfing and to prevent energy loss due to exhaustion. Each group was considered as treatment on attaining 1, 2, 3, 4, 5, 6 and 7 day old age and those for usual 0 day (fresh) as control. Freshly emerged (0 day old) females were given to each male of respective treatment and allowed to mate for 3 hrs. There were 4 replications for each category of treatment and control. For each replication 20 pairs were considered for observation on mating

capacity. While for the study of fecundity and fertility it was based on the date of 12 mated females of each category and control. The computed data of mating capacity, fecundity and fertility were analysed statistically by analysis of variance.

Results and Discussion

The males of 1 and 2 day old mated successfully with fresh females (mating success refers only to the locking of copulatory organs) and no significant difference with control (Table 1).

Table 1. Mean number of male moths mated at 0, 1, 2, 3, 4, 5 and 7 day age and the fecundity and fertility of the mated female.

Male age at mating (days)	Male moths mated (No.)	Fecundity (No.)	Fertility (%)
1	19.50	430	98.49
2	19.25	440	97.82
3	16.00	436	98.32
4	16.00	440	98.44
5	15.25	446	97.63
6	12.00	452	98.32
7	12.25	444	98.10
0 (Control)	19.25	431	98.48
C.D. at 5%	1.54	N.S.	N.S.

N.S. Not significant

The mating capacity of males were affected with the subsequent increase of age from 3 day onwards. The gradual increase of male age resulted in corresponding decrease in mating capacity. There was a high negative correlation (r=-0.907) between age of male and their mating capacity (Table 2). The capacity for mating by the older males were significantly less than younger males. The male moths accounting 80.00, 80.00, 76.25, 60.00 and 61.25% were capable of mating in the age group of 3, 4, 5, 6 and 7 day old respectively against 96.25% in control. The male age at mating did not significantly influence the egg numbers released and its fertility (Table 1) by each mated female. The stimulus received by the female due to mating was not affected by the age of the male. In *B. mori* viable sperms in the female genital tracts induced the nervous activity which inturn, accelerated the oviposition activity of the female (Yamaoka and Hirao, 1971; Yamaoka *et al.*, 1971). In the present investigation, in all the treatments most of the mature eggs were laid within 24 hrs. after mating and this is in full agreement with the result of Fugo and Arisawa (1992) and Paul *et al.*, (1993). The sperms were capable of producing necessary stimulus for early active oviposition in mated female irrespective of age of male partner. That the early initiation of active oviposition behaviour is possible only under the influence of viable sperms and not by any mechanical stimuli applied to the females during mating is further evident from the experiment of Omura (1939) using castrated males of triploid sterile males. The injection of oviposition stimulating substance prepared out of testis, seminalis vesicle and accessory glands into virgin female moths failed to show the active oviposition behaviour (Fugo and Arisawa, 1992). In the present experiment the sperms were viable irrespective of male age, as in none of the cases fertility varied significantly. The increase of male age only delays the ejaculation and movement of sperms in female reproductive organs (Kishorkumar and Paul, 1993).

Table 2. Correlation between age of male moth and mating capacity, fecundity and fertility of mated female.

Partner	Correlation Co-efficient (r)
Age of male moth and mating capacity	-0.907 **
Age of male moth and fecundity	0.199 N.S.
Age of male moth and fertility	-0.028 N.S.

** Significant at P<0.01
N.S. Not significant.

In the preliminary studies, it was observed that *B. mori* males lost their body weight and became weak with the increase of age upto 7 days. The adults did not feed, the loss of weight occurred due to energy expendi-

ture and exhaustion and further accelerated due to high temperature and high humidity because in an environment with hazardous variations, the physiology of insect reproduction is always linked with ecological conditions (Labeyrie, 1978). The poor health and less vigour affect mating capacity of males, whereas the fertilizing power of the sperms remain unaffected. We conclude that in absence of refrigeration facility the male moth can be used safely upto 48 hrs. after emergence and subsequent increase of age only reduces the ability to mate. But ageing of male does not impede sperm transfer if mating is allowed, nor decreases the fertilization ability of the spermatozoa.

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Differential Impact of NSKP Extracts on Nutrition and Reproduction of *Taragama siva* Lefbvre (Lepidoptera: Lasiocampidae)

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Abstract: The antagonistic effect of Neem Seed Kernel Powder (NSKP) with special reference to acetone and methanol extracts on growth rate and nutritional indices such as CI, AD, ECD and ECI by the babul defoliator, *Taragama siva* were evaluated. Host leaves of *Prosopis juliflora, Acacia senegal* and *P. cineraria* treated with 0.05, 0.01 and 0.005% of the extracts were evaluated and analysed in order to assess the growth disruption activity. The adverse effect of NSKP extracts on reproductive potential was also estimated in the present investigation. Of the extracts tested against *T. siva* larvae 0.05% methanolic extract of NSKP showed good detrimental effect on survival, growth and egg production.

Keywords: Taragama, sivanutrition, reproduction, Neem Seed Kernel extract.

INTRODUCTION

The wide range of semio-chemicals found in present day plant is the culmination of the selective pressures exerted by biotic factors, one of which is insect predation. This has resulted in the development of detoxification system which is implicated in protecting herbivorous insects from defensive plant allelochemicals. It is now well established that azadirachtin, the most important phagorepellent of Neem Seed Kernel Powder (NSKP) extracts protect plants against insect attack. Haskell and Mordue (1969) and Bernays & Chapman (1977) indicated that azadirachtin proved to be the most potent antifeedant against such insects as *Locusta migratoria migratorioides* and *Schistocerca gregaria*. The works of Rembold & Sieber (1981), Sieber & Rembold (1983) regarding strong antifeedant activity and its growth inhibiting properties in locusts are well known. The results presented here offers further evidence of the impact of NSKP with special reference to acetone, and methanol extracts on growth rate and food intake by *Taragama siva*, which provide indepth information in understanding the relationship between NSKP extracts treated with *Prosopis juliflora, Acacia senegal* and *Prosopis cineraria* leaves and their biological activity against *T. siva*.

MATERIALS AND METHODS

Nutritional Parameters

Various concentrations of NSKP extracts were tested for growth disruption activity with freshly emerged 3rd instar larvae of *T. siva* on *P. juliflora, A. senegal* and *P. cineraria* leaves. In order to have an understanding of the role of each extract, the best solvent extract of methanol at the concentration of 0.05, 0.01 and 0.005% were chosen on *T. siva* as compared to acetone extract and control. In a feeding chamber freshly moulted third instar larvae of *T. siva* were allowed to feed individually and weight gained was determined every twenty four hours. The control leaf had similar conditions except that the leaf was smeared only with water. Gravimetric method of Waldbauer (1968) was adopted to study the nutritional indices. Third instar larvae from the stock culture were weighed and placed individually in the feeding chamber which contains the known weight of treated leaves. Faecal pellets were carefully separated from the left over leaf weighed and dried to constant weight. Feeding experiments were calculated throughout the larval period starting from the third instar. Consumption index (CI) or feeding rate, approximate digestibility (AD), efficiency of conversion of digested food (ECD) and efficiency

Table 1. Food consumption and nutritional indices of T. siva on methanol and acetone extracts of NSKP treated *P. juliflora* leaves

Parameters	Control	Methanolic extract of NSKP (%)			CD at 5%	Acetone extract of NSKP (%)			CD at 5%
		0.05	0.01	0.005		0.05	0.01	0.005	
Average food consumed/day (mg)	1240	160	230	360	59.84	1040	1150	1190	41.18
Final wt. gain by larva (mg)	580	180	240	310	41.97	413	428	472	25.49
Average wet wt. of faces/day (mg)	383	40	75	150	77.48	261	292	312	80.32
CI	1.40	0.24	0.34	0.57		1.18	1.37	1.40	
AD (%)	60.80	75.0	67.30	62.50		76.0	74.6	73.70	
ECD (%)	67.50	15.0	15.40	13.70		49.8	49.8	53.70	
ECI (%)	48.30	112.0	104.00	86.00		37.9	37.2	39.60	

Table 2. Food consumption and nutritional indices of *T. siva* on methanol and acetone extracts of NSKP treated *A. senegal* leaves

Parameters	Control	Methanolic extract of NSKP (%)			CD at 5%	Acetone extract of NSKP (%)			CD at 5%
		0.05	0.01	0.005		0.05	0.01	0.005	
Average food consumed/day (mg)	1123	137	195	852	19.82	823	917	1021	8.59
Final wt. gain by larva (mg)	518	148	205	362	23.29	358	392	423	19.78
Average wet wt. of faces/day (mg)	372	35	67	120	16.58	205	252	291	8.51
CI	1.23	0.19	0.25	1.02		1.17	1.27	1.38	
AD (%)	66.87	74.40	65.64	85.92		75.09	72.52	71.49	
ECD (%)	68.97	112.12	160.16	49.45		57.93	58.95	57.94	
ECI (%)	46.13	108.03	105.13	42.49		43.49	42.75	41.42	

Table 3. Food consumption and nutritional indices of *T. siva* on methanol and acetone extracts of NSKP treated *P. cineraria* leaves

Parameters	Control	Methanolic extract of NSKP (%)			CD at 5%	Acetone extract of NSKP (%)			CD at 5%
		0.05	0.01	0.005		0.05	0.01	0.005	
Average food consumed/day (mg)	925	513	625	716	17.34	723	862	902	6.01
Final wt. gain by larva (mg)	317	169	183	285	1.52	251	267	300	12.53
Average wet wt. of faces/day (mg)	259	125	180	200	2.75	158	215	381	20.91
CI	1.19	0.60	0.89	0.95		0.99	1.17	1.19	
AD (%)	72.0	75.63	71.22	72.07		78.15	75.06	57.76	
ECD (%)	47.59	43.56	41.12	55.23		44.43	41.27	57.58	
ECI (%)	34.27	32.94	29.28	27.93		34.72	30.97	33.26	

Table 4. Impact of methanol and acetone extract of NSKP treated leaves of the host plants on the reproductive efficiency of *T. siva*

Treatments	Mean fecundity					
	Methanolic extract of NSKP (%)			Acetone extract of NSKP (%)		
	P. juliflora	*A. senegal*	*P. cineraria*	*P. juliflora*	*A. senegal*	*P. cineraria*
Control	133.10	110.20	95.20	133.10	110.20	95.20
0.05%	69.62	65.12	42.13	111.50	85.30	80.20
0.01%	86.00	74.29	62.10	122.70	84.20	82.30
0.015%	103.00	95.21	83.56	128.90	97.10	90.80
CD at 5%	5.75	5.87	3.11	1.36	4.25	4.82

of conversion of ingested food (ECI) were estimated from the above experiments.

Fecundity studies:

A group of larvae were selected from the abovesaid treatments of methanol and acetone extracts. Fresh leaves of *P. juliflora, A. senegal* and *P. cineraria* treated with 0.05, 0.01 and 0.005% were provided inside the container without allowing the larvae to starve, throughout the experimental period. From this a pair of male and female moths were selected to study the adverse effect of NSKP extracts on reproductive potential of *T. siva* and to estimate the number of eggs laid throughout the life time of the test insect was recorded and replicated five times. All the results were subjected to analysis of variance and the differences of food intake, nutritional indices by *T. siva* on various host leaves treated with NSKP--methanol and acetone extracts were correlated at 5% level.

RESULTS

Methanol and acetone extracts of NSKP on *P. juliflora* leaves

An analysis of food consumption and the relative influence on nutritional indices indicated clear variation when fed on *P. juliflora, A. senegal* and *P. cineraria* leaves treated with methanol and acetone extracts at the concentration of 0.05, 0.01 and 0.005%. The results of impact of NSKP extracts on the host leaves and their utilization experiments are summerized in tables (1 to 3). The amount of food ingested by *T. siva* was least on the host leaves treated with 0.05% of methanol extracts and significantly different from that of acetone extracts and control. This difference may be due to the variation in the nutritive contents of the food; weight loss by larvae, rate of development, quality of food consumed, approximate digestibility and efficiency of food utilization reflecting the nutritional adequacy of the diet. Nutritional indices were estimated for *P. juliflora* leaves treated with methanol and the results showed that *T. siva* larva ingested 160 mg/day of food at higher concentration (0.05%) as compared to control (1.24 gm) and the rate of ingestion increased at the lower concentrations. A closer look on the weight gain of the larva also showed that larvae fed on the leaves treated with 0.05% of NSKP-methanol extracts had a poor weight gain as compared to control, acetone extracts and other lower concentrations. At the lowest concentrations of methanol and acetone extracts, the larvae excreted more, while at higher concentrations the faecal pellets egested was low probably because of poor consumption. The consumption index (CI) being higher in control as well as in all the concentration of acetone extracts and considerably reduced in methanolic treated individuals. The higher AD, ECD and ECI values in treatments compared to control could presumably be attributed to the fact that the larvae defaecate excessively the leaves containing toxic substance in a physiological event to remove the toxic substance and hence relatively less quantity of food material is allocated to body tissue.

Methanol and Acetone extracts of NSKP on *P. cineraria* leaves

A totally different picture was obtained when individuals of *T. siva* were subjected to *P. cineraria* leaves treated with methanol extracts. The nutritional indices and related parameter showed that almost similar quantity of diet was ingested as that of control at higher concentrations except 0.05% of methanol extracts (Table 3). The CI, ECD and ECI were also significantly much lower in all the concentrations including control as compared to *P. juliflora, A. senegal* leaves (Table 2) treated with methanol. The inhibition of ECD and ECI caused a marked reduction in the overall efficiency of injected food. A significant difference in the final weight gain was observed between control and various experimental individuals at different concentrations with that of the previous experiments. AD of *T. siva* remained high when fed with higher concentrations, especially in the case of *P. cineraria* leaves is compared to *P. juliflora, A. senegal* and control which reflects the attempt by the

insect to compensate for reduced consumption and utilization of treated leaves in order to maintain the growth.

Impact of NSKP extracts on fecundity

The reproductive ability of moths emerging from larvae fed on leaves treated with methanol extracts greatly reduced as compared to acetone treated and control. Among the extract tested, *P. cineraria* leaves with 0.5% level greatly inhibited egg production than others (Table 4). The fecundity of *T. Siva* showed marked variation in various host plants and high fecundity was observed in control (133.1) followed by *A. senegal* (110.2) and *P. cineraria* (95.2), the variation appeared to be due to the efficiency, with which digested food gets converted into body material.

DISCUSSION

The present investigation considerably substantiates the hypothesis that the natural plant products like NSKP may have chronic effects on rate of growth, ingestion and utilization of food by herbivores. Nutriional index experiments showed that methanol extracts had adverse effect on any of the nutritional indices, but still tend to affect growth especially at higher concentrations. Added to this, acetone extracts did not exert much adverse effect and normal growth. The differences in the timing of mortality following different concentrations closely linked with slower growth of the insect and lack of feeding (Kogan & Paxton, 1983). With the increase in concentration food intake gets reduced, growth becomes slower and moultng is inhibited in *Heliothis armigera* as well as in *Spodoptera litura* (Murugesan & Jacob, 1994). The amount of food ingested and weight gain by *T. siva* were markedly reduced on *P. cineraria* leaves treated with 0.05% methanol extract of NSKP compared to other hosts and control, when fed on different concentrations. The ECI and ECD into body mass was considerably reduced compared to control. Fagoonee (1984) reported similar results in *Crocidolmia binotalis* with azadirachtin treated diet. As indicated by Mark & James (1988), in *M. virescens* the reason for the low utilization efficiency in *T. siva* at higher concentrations may be due to the dissipation of energy from the production of body mass to detoxification. AD of *T. siva* remained high when fed with higher concentrations compared to control and acetone extracts, reflects the attempt by the insect to compensate for reduced consumption and utilization of treated leaves in order to maintain the growth. Similar results were also observed by Fragoonee (1984) in *C. binotalis*. This study also showed that the facundity of emerging moths after NSKP treament was greatly reduced. Patterson (1978) examined the effect of larval nutrition on egg production by injected proteins, particularly organic nitrogen, and proteins are well known for growth, survival and fecundity of insects (Slansky, 1982). At higher concentrations, the plant products like NSKP reduced egg production and also hatching percentage (Brattsen, 1983). It is also well known fact that several plant products not only affect the survival and growth of the herbivores, but also make them available to natural enemies for a longer period and raises the probability of mortality. The foregoing results therefore, clearly indicate the adverse effect of NSKP methanol extract at 0.05% induce physiological changes that may reduce the nutritional indices, survival and growth.

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BRIEF COMMUNICATION

Spermatogenesis in the Testis Implanted into Female Larvae of *Spodoptera mauritia* Boisd. (Lepidoptera: Noctuidae)

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Keywords: Spermatogenesis, *Spodoptera mauritia*, implantation, testis, female larva, hormones.

(Received in September 1995)

Abstract: Testes of fifth and sixth instar larvae of *S. mauritia* were implanted into female larvae of the same age group. Tests were relaimed in day 1 pupa and the effects on testicular development and spermatogenesis were studied. Reclaimed testes showed an increase in volume, eupyrene and apyrene spermatogenesis. However the number of different types of spermatocysts was considerably less when compared to testes kept as control. The significance of these findings is discussed.

The available information on the regulatory factors in insect spermatogenesis is incomplete and contradictory. On the one hand it has been proposed that in insects spermatogenesis may proceed as a sequential autodifferentiative process which is not affected by the alterations in the titre of morphogenetic hormones or other factors (Lender and Deveau-Hagege, 1963; Loeb *et al.*, 1985). On the other hand a vast amount of literature has accumulated implicating hormones in the regulation of spermatogenesis (reviewed by Dumser, 1980; Koeppe *et al.*, 1985). In lepidopteran larvae, the testes lie somewhat freely in the body cavity as paired structures loosely attached to the body wall by means of trachea and nerve fibres. This facilitates easy removal of testes and hence lepidopteran testes has been a convenient experimental material for *in vivo* and *in vitro* studies on the role of hormones and other factors in spermatogenesis (Takeuchi, 1969; Kambysellis and Williams, 1971; Kiss and Williams, 1976; Friedlander and Benz, 1981; Jans *et al.*, 1984). In *Spodoptera mauritia* Boisd (Lepidoptera: Noctuidae) spermatogenesis is initiated in the penultimate larval instar. In the last larval instar gonial cells undergo meiosis to form spermatocytes which are bipotential producing first eupyrene (nucleate) and later apyrene (anucleate) spermatozoa (Venugopalan *et al.*, 1994). In this paper we have made an attempt to study whether the regulatory factors in the spermatogenesis of *S. mauritia*, are sex determined or not, by implanting testis into female larvae and then studied the progress of spermatogenesis.

Day 1 fifth (penultimate) and sixth (last) instar larvae of *S. mauritia* were segregated from laboratory stock culture reared and maintained as described earlier (Nair, 1981). One lobe of the paired testes of day 1 fifth/sixth instar larvae was implanted in to day 1 fifth/sixth instar female larvae respectively. Recipients and donors were anaesthetised with diethyl ether. Donor testis was dissected in sterile insect Ringer solution and implanted into the host through a slit made on the abdominal tergite. One lobe of the testis of day 1 fifth/sixth instar larvae was freed from nervous and tracheal connections and kept as control. The wounds were surface sterilized with alcohol and smeared with a mixture of phenylthiourea/streptomycin sulphate. The im-

planted/ control testis were reclaimed in day 1 pupa and volume calculated. Volume of implanted/ control testis was calculated assuming the testis to be an ellipsoid using the formula $V=\pi/6$ length $\times$ width^2. Reclaimed testes were fixed in Bouin's fluid and processed for routine histological studies. Histologically the spermatocysts were differentiated into 5 developmental stages (1) spermatogonial cysts (2) spermatocyte cysts (3) spermatid cysts (4) eupyrene sperm bundles and (5) Apyrene sperm bundles. The number of testicular cysts for each type was counted in two sections having the maximal surface area from among serial sections of five independent samples. Each data point thus represent the mean (± SD) value taken from 10 measurements.

The testicular volumes of fifth and sixth instar larvae at the time of implantation were 0.73 ± 0.16 mm and 1.30±0.46 mm respectively (see Table 1). The volume of testis reclaimed from day 1 female pupa shows a considerable increase in size. Testis volume approximately doubled. The volume of testis kept as control however showed a four fold increase in its dimensions.

Table 1. Changes in the volume of testis implanted into female larvae

Experiment	n	Volume of testis in mm (mean ± SD)	
		At the time of implantation	Reclaimed from pupa
Testis implanted into fifth instar larvae	34	0.73±0.16	2.50±0.82
Control	10	0.73±0.16	4.49±0.35
Testis implanted into sixth instar larvae	35	1.30±0.46	2.45±0.13
Control	10	1.30±0.46	4.49±0.35

Of major interest in this paper is the finding that histological preparations of testis implanted into female larvae showed the presence of all types of spermatocysts. However the number of different types of spermatocysts is considerably less when compared to the testis kept as control (see Fig. 1). Regulatory factors involved in lepidopteran spermatogenesis are far from clear. A few studies have demonstrated that dichotomous spermatogenesis is promoted by two blood borne factors: eupyrene spermatogenesis inducing macromolecular factor (Kambysellis and Williams, 1971a; Kiss and Williams, 1976) and apyrene spermatogenesis inducing factor (Leviatan and Friedlander, 1979; Friedlander and Benz, 1981; Jans *et al.*, 1984).

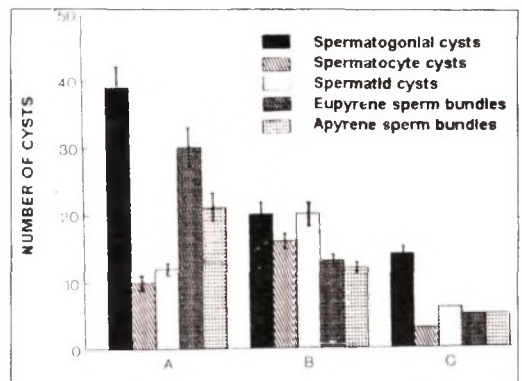

Fig. 1. Testicular spermatocyst count in day 1 pupa Control testis (A), Testis after implantation into female sixth (B) and fifth (C) instar larva

In many insect species the role of ecdysteroids in promoting spermatogenesis has been well documented *in vivo* (Takeuchi, 1969; Dumser and Davey, 1975) and *in vitro* (Schmidt and Williams, 1953; Yagi *et al.*, 1969; Kambysellis and Williams, 1971b, 1972; Fukushima and Yagi, 1975) Further several investigators have shown that the testis contain considerable quantities of ecdysteroids and these are secreted by testis sheath (Koolman *et al.*, 1979; Loeb *et al.*, 1982; Gelman *et al.*, 1985, 1989). Ecdysteroids stimulate mitotic and/or meiotic divisions during early stages of spermatogenesis (Yagi *et al.*, 1969; Kambysellis and Williams, 1971b, 1972; Takeda, 1972; Dumser and Davey, 1975), interact with haemolymph macromolecular factor to induce spermatocyte differentiation (Kambysellis and Williams, 1971b, 1972) and influence the development of apyrene sperm (Hoffmann and Behrens, 1982;

Loeb *et al.*, 1982; Gelman *et al.*, 1989). The results of the present study suggest that the spermatogenesis inducing haemolymph factors hormonal or otherwise are not sex determined. Further it may be speculated that the lower number of spermatocysts in the implanted testis is caused by the low titre of ecdysteroids in the female larvae of *S. mauritia*. A few studies in lepidopterans have demonstrated a sexual dimorphism in ecdysone titre during gonadal development (Hsiao and Hsiao, 1977; Bollenbacher *et al.*, 1978; Bodnaryk, 1986). In *Mamestra configurata* ecdysteroid concentration in males is greater than that of females (Bodnaryk, 1986).

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ENTOMON 1995 VOL. **20** NO. 3 & 4: 267-269

BRIEF COMMUNICATION

On a New Species of *Ujna* Dist. (Homoptera: Cicadellidae) with a Note on *Ujna delicatula* Dist.

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Received in October 1993

Abstract: A new species of *Ujna* Distant namely *U. bicolorata* is described and illustrated. *Ujna delicatula* Dist. is also reported here from India for the first time.

Key words: Ujna Dist., *Ujna bicolorata*, *Ujna delicatula*

Ujna Dist. is a small genus known by six species and represented by Oriental and Malagasian Regions (Oman *et al.*, 1990). One of them, *Ujna, flavidipes* Dist. is known from Mahe islands, while from Sri Lanka, three species are reported. They are *Ujna delicatula* Dist., *U. exigua* (Melichar) and U. *gagatina* (Melichar). From Burma *U. consors* Dist. is known while from the Philippines, *U. philippines* Baker is reported (Distant, 1908; Metcalf, 1965). While studying the leafhoppers from Tamil Nadu the author came across a new species and the same is described here. *U. delicatula* Dist. is also reported here for the first time and it's male genitalia which has not so far been studied, is also described.

Ujna delicatula Distant

Ujna delicatula Dist., 1908, *Fauna Brit. India, Rhynchota,* IV: 239.

Ujna delicatula dist. Metcalf, 1965, *General catalogue* of *the Homoptera.* vi(1), *Tettigellidae*, 296.

Male genitalia: (Fig. 1-3)

Pygofer broad at middle, it's process arising ventrally and not reaching posterior margin at caudal end. Male plate long, broad at base and slightly tapering towards apex with numerous setae from beyond middle. Style narrow, elongate, pre-apical lobe well developed and pointed, apophysis elongate with a hook at the mesal margin near middle. Connective Y-shaped, arms separate, stem long. Aedeagus broad at base cylindrical, shaft short and narrow.

Measurements

Male 3.52 to 3.68 mm long and 0.72 to 0.80 nm wide.

Material examined

India: Tamil Nadu: Periyar Dist., Dhimbam, 840 m. 2. ♂ ♂, 1-1-1990, Coll. P.T. Cherian.

Remarks

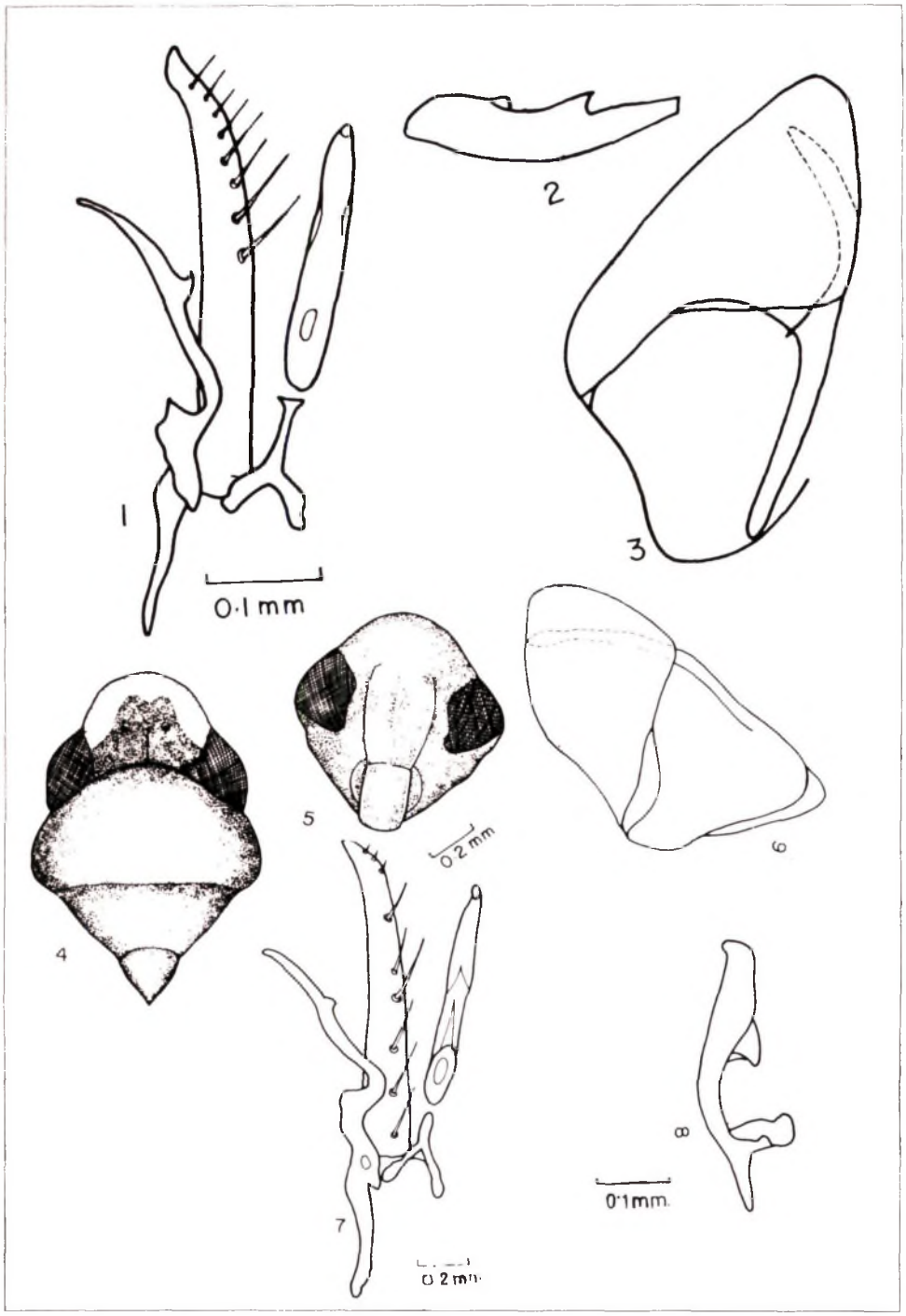
U. delicatula is a reported for the first time from India.

Ujna bicolorata sp. nov. (Fig. 4-8).

Vertex broadly rounded, pale ochraceous at apex and upper regions of eyes: base of vertex to ocelli castaneous, and extended upwards like an incomplete dome: a fine central line from ocelli to base black. Face longer than broad, without spot at base; frontoclypeus pale ochraceous; lora small, not reaching apex of clypellus. Pronotum and scutellum chocolate brown, the latter with fine transversely arched impression. Forewing brownish, costal area interrupted beyond middle and devoid of any oblique lines at apex.

Male genitalia

Pygofer broad at base, it's process reaching posterior margin at caudal end. Male plate long



Figs. 1-3. *Ujna delicatula* Dist. (1) Male plate, Style, Connective, Aedeagus-dorsal view; (2) Aedeagus lateral view; (3) Pygofer-lateral view. Figs. 4 - 8. *Ujna bicolorata* sp.nov. (4) Head and Thorax-dorsal view; (5) Face-ventral view. (6) Pygofer-lateral view; (7) Male plate, Style, Connective, Aedeagus-dorsal view; (8) Aedeagus-lateral view.

with setae arising from near base. Style long, pre-apical lobe well developed and blunt, apophysis long, curved laterad and with blunt projection at middle, base of style elongated, narrow and with small hole near arm of connective. Connective Y-shaped, arms widely separated. Aedeagus long, base slightly curved, dorsal apodeme moderately developed, shaft narrow and elongated.

Measurements

Male 4.32 mm long and 0.84 mm wide.

Material examined

Holotype, ♂, Tamil Nadu: Periyar Dist., Dhimbam, 1-1-1990, Coll. P. T. Cherian.

Remarks:

Ujna bicolorata sp. nov resembles *U. consors* Dist. in general colouration of vertex bit differs from it in the absence of spots and lines on the vertex and of oblique brown line at costal margin beyond middle. It differs from all other species by its distinct bicolourous nature of its vertex, its characteristic style having a hole near connective and pygofer process reaching posterior margin at caudal end.

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BRIEF COMMUNICATION

Additional Records of Insect Pests of Pulses in South Andamans

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Received in December 1994

Key words : insect pets Lepidoptera, Pulses, South Andamans

Pulses are usually grown in the dry season (summer) after the harvest of paddy crop in a limited area of 621 ha. in the Andaman and Nicobar islands (Anonymous, 1987). Earlier 24 species of pests attacking pulses were reported from the Andaman islands (Anonymous, 1983; Bhumannavar 1990a; 1990c; 1992).

The insect pests collected during periodic surveys mainly in rabi season (1991-1993) on different pulse crops were brought to the laboratory and reared for adult emergence. Later they were sent to the International Institute of Entomology for confirming their identities.

A total of ten additional species of lepidopteran pests were collected during the survey.

Neptis hylas L. (Nymphalidae: Lepidoptera): The body of the straw coloured larva measuring 3 cm long is wrinkled with four pairs of dorsal tubercles, and an yellowish white band running all along the dorsal surface from the third to the last abdominal segment. The larvae feed on tender terminal leaves of red gram but do not cause significant damage.

Pingasa ruginaria Guenee (Geometridae: Lepidoptera): The cryptic larva prefers feeding on tender leaves and can easily be mistaken for unopened axillary bud. They are minor pests of red gram, causing negligible damage.

Hyposidra talaca Walker (Geometridae: Lepidoptera): A green looper, which on maturity turns pinkish, resembles very much the twig of the plant, measures up to 6 cm in length. It causes moderate damage to red gram. This is also reported to attack millets, castor, sweet potato, jamun, mango, rose, agathi (Nair, 1975), leaves of *Citrus medica* L. and inflorescence of cashew (Bhumannavar, 1990b). *Omiodes dimenalis* (Guenee) (Pyralidae: Lepidoptera): Greenish larva, measuring 0.5-0.8 cm in length, folds the tips of red gram leaves and feeds on the green matter from within. The damage noticed is moderate in red gram. It has also been found to damage horse gram (personal observation) in Andamans. Yet another species *O. indicuta* F. was found to damage french bean in the Andaman islands.

Mocis undata F. (Noctuidae: Lepidoptera): A defoliator, the larva makes holes on the red gram leaves and is confined to the ventral surface.

Anticarsia irrorata F. (Noctuidae: Lepidoptera): The larva is pale green and stout, a defoliator, measuring upto 6 cm in length, seen along the petiole of the compound leaf of cowpea.

Homona permutata Meyrick (Tortricidae: Lepidoptera): A green larva, measuring 0.8 cm in length, rolls the leaves of red gram and feeds on green matter from within, causing minor damage. This species is also known to damage *Citrus medica* L. It webs the leaves of mango and guava in South Andaman (Bhumannavar, *et al.*, 1991).

Dasychira mendosa (Hubner) (Lymantridae: Lepidoptera): This hairy caterpillar measuring 5cm long prefers tender leaves of red gram and cowpea. This is known to attack castor, banana, mango, apple, peach, coffee and tea in mainland (Nair, 1975). In South Andaman this is an important pest of mango (Bhumannavar, 1990b) and has been recorded as feeding on leaves of cinnamon (Veenakumari and Prashant Mohanraj, 1993).

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ENTOMON 1995 VOL. 20 NO. 3 & 4: 273-275

BRIEF COMMUNICATION

Predation of the Subabul Psyllid *Heteropsylla cubana* by the Drgonfly *Pantala flavescens* in Nature

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Abstract: Swarms of the dragonfly *Pantala flavescens* were found to systematically predate on the subabul psyllid pest *Heteropsylla cubana*. In a more active manner of feeding, individual dragonflies in hovering flight very close to the subabul shoots, create strong air currents which appear to flush out some of the adult psyllids which are then predated upon. The level of predation effected appeared to be considerable enough to have a detectable impact on the numbers of the pest. This is perhaps the first record from India of the sustained predation of any agriculturally important insect pest species by dragonflies.

Key words: Predation, Subabul psyllid, *Heteropsylla cubana*, dragonfly, *Pantala flavescens*

Both the dragonflies (*Anisoptera*) and the damselflies (*Zygoptera*) of the Order *Odonata* are exclusively insectivorous. The dragonflies perhaps have the most well developed adaptations for predatory life: viz., for locating, chasing, capturing and devouring the prey while in flight. Although their prey are generally small like the *Culicidae, Chironomidae*, other dipteran flies, small lepidopterans, homopterans, etc., some of the larger dragonflies capture and consume medium-sized insects, especially the weak fliers like the swarming alates of termites (*Isoptera*) and the damselflies. Corbet (1980) observed that as general and opportunistic predators, the dragonflies seldom diminish a prey population enough to be of obvious economic value. However, Shortenberger (1967) found that in western Tanzania 12 species of dragonflies regularly from intra-species feeding aggregations and that they probably constitute the principal biological factor affecting the breeding success of the Red Locust, *Nomadacris septemfasciata*.

The multi-purpose Subabul tree, *Leucaena leucocephala* (Lam.) de Wit. provides fodder, fuel, organic manure and timber in many tropical countries including India. With the realisation that this wonder tree can provide better nourishment for our improved stocks of cattle and play an important role in augmenting the fodder resources of our country, Subabul plantation has been taken up in a big way in the different agro-climatological zones in India, including the dry zones. Unfortunately in recent years the Subabul trees grown in Kerala, other regions in India and several other countries, are severely attacked by the psyllid pest *Heteropsylla cubana* Crawford (Fig. 1). Large numbers of adults of this psyllid infest almost all the tender shoots of each tree and lay numerous eggs on each leaf. The feeding aggregations of nymphs (Fig.2) of different stages and the adults on the shoots retards their further growth. In severe cases of attack the leaves become chlorotic and undersized; ultimately the branches become defoliated and dry up as a result of die back syndrome. As its life-cycle is short, this psyllid is capable of infesting successive flushes of foliage under favourable weather conditions. Thus the infestation can be continuous through the year except during the monsoon months.

In the Dhoni Bull Farm of the Kerala Livestock Development Board Ltd., in Olava-

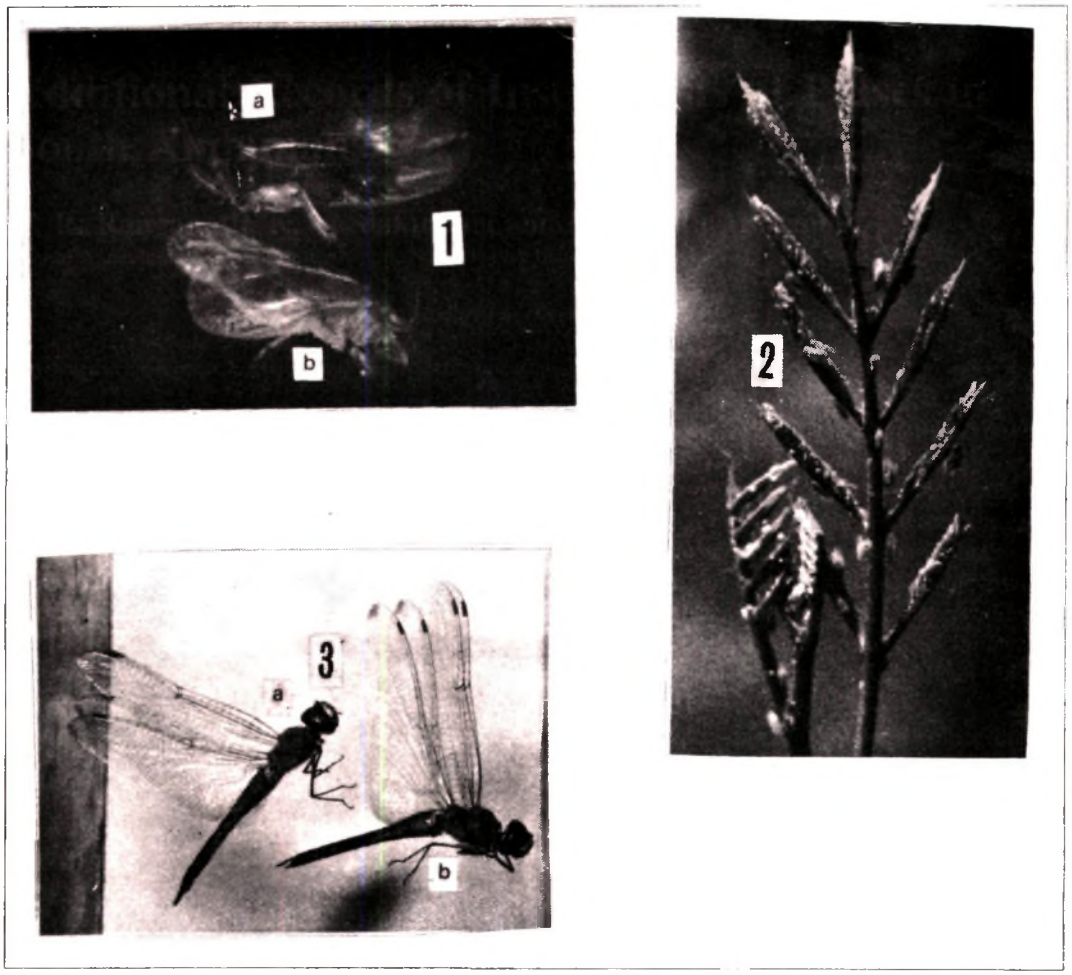

Figure 1. The subabul psyllid, *Heteropsylla cubana*. a: female; b: male.; Figure 2. A tender subabul shoot infested by aggregations of adults and nymphs of *Heteropsylla cubana*.; Figure 3. *Pantala flavescens*, the dragonfly predator of the subabul psyllid. a: female; b: male.

kode, Palakkad District, where some 4000 subabul trees are grown under the seed production and animal feed programmes, extensive damages were reported during the last four years due to large scale infestation by *H. cubana*. the maximum psyllid population build up during 1992 was found to take place during the two month period beginning from the third week of August.

It was precisely during this two month period of the year that the maximum population build up of the fairly large-sized, robust and communal dragonfly *Pantala flavescens* (Fabricius) (*Libellulidae*) (Fraser, 1936) (Fig.3) was observed in the Dhoni Farm area, as a result of the congregation of hordes of adults of this species simultaneously emerging from the water bodies in the region. Hundreds of swarms, each formed of 50-200 individuals of both the sexes of this dragonfly were seen flying and hovering all around and above the subabul trees, apparently chasing, capturing and devouring the adult psyllids flattering from one shoot to the other.

Individuals of *P. flavescens* were also found to feed on adult psyllids on subabul shoots in

a more active and ingenious manner. Chopard (1949) states that the hovering flight of odonates produces strong air currents directed downwards. Such air currents produced by individuals of *P. flavescens* during their hovering flight very close to the subabul shoots appeared to
disturb and flush out some of the adult psyllids which flew up, only to be predated upon by these attendant dragonflies.

It was also noted that whenever an infested subabul shoot was bent down for close observation *in situ*, 2 or 3 individuals of *P. flavescens* flew in on the scene without being in the least inhibited by the presence of the observers, and predated on the psyllids that flew up on account of the disturbance caused.

It is reasonable to conclude from the above mentioned observations that the level of predation of the subabul psyllid effected by the dragonflies in the Dhoni Farm during the said two month period each year must be very considerable and that it must have a detectable impact on the numbers of this pest.

This appears to be the first record from India of the systematic predation sustained over a two month period, of any agriculturally important insect pest species by dragonfly swarms. Joseph & Lahiri (1989) and Miller (1989) have found that individuals on such feeding aggregations do remain as roosting aggregations by night during a period of more than two months. In view of the remarkable ability of seasonal swarms of dragonflies in appreciably suppressing the numbers of important pest species, it is considered high time that we revise the status given to these arch predators by FRASER (1933) as scavengers of the atmosphere destroying noxious flies and mosquitoes as well as the smaller moths which are regarded as pests, to the higher status of economically very important polyphagous and opportunistic predators. By taking adequate steps for conserving the species diversity and abundance of our dragonfly fauna, we definitely can gain much from the natural biological suppression effected by these important predators in any integrated pest management programme in which their role has relevance.

ACKNOWLEDGEMENTS

The authors wish to place on record their thanks to Mr. P. Girijavallabhan, Coordinator, Dhoni Farm, Palakkad, Kerala, for giving facilities for our field work and for various other courtesies. One of us (KJJ) is grateful to the Chairman, State Committee on Science, Technology & Environment, Trivandrum, for financial assistance.

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ENTOMON 1995 VOL. **20** NO. 3 & 4: 277

BRIEF COMMUNICATION

Simple New Killing Bottle for Entomologists

A. M. Renjith & D. Sitaramarao
College of Horticulture, Vellanikkara. P. O. 680 654, Kerala

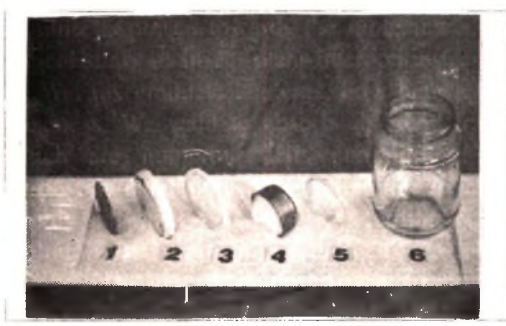
Received in December 1994

Abstract: A new type of killing bottle for insects which can easily be made, and useful for collecting insects without any damage to them is described.

Key words: Killing bottle, delicate insects

Entomologists the world over have relied for a long time on the killing bottle using cyanide for collecting insects. The chemicals included calcium cyanide, sodium cyanide or potassium cyanide. Currently however, this device is not very common, due to the extreme toxicity of cyanide, the cumbersome procedure in making the bottle and the precautions to be taken in handling the same. There is also another drawback for the killing bottle using cyanide because they sweat inside, when exposed to bright sunlight. Presently, insect killing bottles are made with a swab of cotton dipped in chloroform, ethyl acetate or any other anaesthetic. Killing bottles made this way can be used instantly, and the anaesthetic poses lesser hazards. However, while collecting small insects such as psyllids, leafhoppers, plant hoppers and whitefly adults, they get stuck to the cotton padding. The anaesthetic also wets the collected specimens. Even butterflies and moths are wetted, causing discolouration of the scales.

We devised a simple but efficient killing bottle which overcame these difficulties. The bottle used is a conventional jam bottle, with a metallic cap, preferably a half-screw closing type. An exploded view of the killing bottle is given as Fig.1. The lid of the bottle is cut in the shape of a wedge at three points and pushed inwards (Fig.1, no. 2). The plastic/ rubber cushioning inside the lid (no. 3) is cut as a ring to accommodate a burner cap (no. 4) used in kerosene stoves. Cotton is packed inside the burner cap and pressed onto the metallic lid with the three wedges clipping on to it. An additional plastic washer inserted from below will fix the burner cap onto the lid further. The gap formed by the cutting of the lid of the bottle is sealed by pasting a round metallic sheet over the lid (Item no. 1 Fig. 1). Chloroform or any other anaesthetic is poured into the cotton padding and pressed to the roof of the lid and the bottle closed. The killing bottle is now ready for use. The vapours of chloroform spreads into the whole of the bottle and insects can be collected by opening the lid and directing the killing bottle on them. They are collected at the bottom of the bottle, with no discolouration to their wings or any other mechanical damage. When the vapours of the anaesthetic are exhausted, it can be poured through the holes on the burner cap fixed to the lid, and this way, it is not necessary to open the lid setting at any time. The anaesthetic is absorbed by the cotton padding.



We found the new type of killing bottle very useful in collecting aphids, psyllids, bugs, microlepidopterans and a variety of other insects.

ENTOMON 1995 VOL. 20 NO. 3 & 4: 279-281

BRIEF COMMUNICATION

Development of an Artificial Diet (Semi-Synthetic) for Rearing Armyworm *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) Using Flour of Different Commodities

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Received in August 1994

Abstract: A semi-synthethic diet has been formulated for mass culture of *Mythimna separata*. Different parameters like larval pupal period, percent pupation, percent adult emergence, physical condition of the adult have been studied. Results indicated that *M. separata* can be successfully mass-reared in the laboratory on the french bean flour based diet.

Key words: Armyworm, Artificial diet, *Mythimna separata*.

Maize crop is attacked by a number of insect pests during different seasons, (Sharma, 1984; Ragu and Sharma, 1988), *M. separata* being one of the major pests.

Research on any aspect of Entomology will begin with culturing insect colonies which are used as test insects, such colonies must be of uniform nature in all aspects.

Artificial diets provide colonies containing insects of known age group, thus formulation of artificial diet is a pre-requisite.

Taking *M. separata* as the test insect, it was decided to develop a semi-synthetic diet using flour of 16 different cereals and pulses.

The basic composition of the diet was adopted from Tiwari and Bhattacharya (1987). In all sixteen different commodities in flour form was used to select out the best formulated diet.

Composition of the diet was as below:

Commodity	17.67 gm.
Yeast powder	3.07 gm.
Sodium Ascorbate	0.31 gm.
Sorbic acid	0.15 gm.
Methyl-p-hydroxy benzoate (Methyl paraben)	0.31 gm.
Agar	1.54 gm.
Formaldehyde 10%	0.15 ml.
Water (distilled)	76.80 ml.

Different commodities used in flour form were bajra, blackgram, cow-pea, french-bean, gram, green-gram, lentil, maize, pea, ragi, red gram, rice, sorghum, soybean, wheat, winged-bean. All the commodities were cleaned and sundried and then ground to flour form.

Flour of the above commodities was transferred to a blender and mixed thoroughly for two minutes with half quantity of distilled water to get a homogenous mixture. Yeast powder, methyl paraben, sorbic-acid and formaldehyde were added to the above ground commodity in the blender and mixing was continued for 2½ minutes.

Agar was dissolved in the remaining half quantity of water, the water was boiled and agar was added slowly and stirred continuously, and then cooled at room-temperature and added to the blender, to the contents of the mixture, and the entire diet was mixed thoroughly. Finally ascorbic acid was dissolved in a little amount of water and added to the formulating diet, and the blending process continued for 2-3 minutes.

The homogeneous diet was transferred immediately into plastic vials, (15× 5cm)

Table 1. Developmental profile of *Mythimna separata* on diets prepared with flour of different commodities

Commodity	Larval period (days)	Pupal period (days)	Percent Population	Percent adult emergence	Physical condition of adults
Bajra	18.00	12.66	48.86 (44.3)	26.66 (31.0)	N, Wex
Blackgram	14.00	11.83	54.99 (47.9)	36.66 (37.2)	Ww
Cow-pea	22.50	--	--	--	--
French bean	18.33	10.33	84.40 (66.7)	81.10 (64.2)	N
Gram	26.33	11.55	56.66 (48.7)	46.66 (43.0)	N, Ww
Greengram	16.33	12.66	54.44 (47.5)	44.40 (41.7)	N, Wex, Ww
Lentil	31.22	22.11	32.10 (34.5)	13.33 (21.3)	Wex
Maize	25.00	--	--	--	--
Pea	16.33	12.33	57.70 (49.4)	24.44 (29.6)	Abem
Ragi	17.33	11.33	36.44 (37.1)	18.66 (25.55)	Ww
Rice	22.60	9.44	48.66 (44.2)	36.66 (37.2)	Wex
Redgram	17.11	14.33	74.00 (59.3)	59.99 (50.7)	N, Ww
Sorghum	25.33	13.66	24.20 (29.4)	8.86 (5.38)	D, Ww
Soybean	38.50	--	--	--	--
Wheat	25.00	--	--	--	--
Winged Bean	--	--	--	--	--
Control	19.00	10.00	90.00 (71.5)	80.00 (27.6)	N
S.Em. ±	2.229	0.666	0.2067	2.221	
C.D. at 5%	6.423	1.937	0.6010	6.515	

Data in parentheses indicate - All died.
angular transformed values, N = Normal adults, Wex = Adult wings not expanded, Ww = Adults with wavy wings, Abem = Adults failed to come out of pupalcase, D = deformed

allowed to cool at room temperature and stored at 4°C until used.

The newly hatched first instar larvae (0-12 hours old) were used in all experiments.

A small amount of the diet was placed on the inner wall of the vial (20 × 12 cm) with a screw cap provided with a hole and a fine brass mesh for aeration).

Ten larvae were released in each vial, diet was changed at 4 day interval in the beginning and at alternate days during later stages. After 8th day larvae were reared individually for comparing the growth and development of insect on natural and semi-synthetic diets, larvae were also reared on maize leaves as control.

Comparison between different formulated semi-synthetic diets was made based on larval period, pupal period, percent pupation, percent adult emergence. Adult deformities were also recorded.

Among the sixteen diets tested, french bean diet was found best among all flour based diets. Red gram was next in order followed by gram, green gram and bajra.

French bean diet was best as it supported

maximum larval survival. Larval and pupal period on french bean were equal to control. It showed maximum number of adult emergence which were normal, without any deformity.

On red gram both normal and abnormal adults were recorded. Cowpea, soybean and winged bean based diets were found poor. The present investigation revealed that french bean flour based semi-synthetic diet was successful (nearly equal to control) for rearing large number of *M. separata* larvae for control programme or screening.

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ENTOMON 1995 VOL. 20 NO. 3 & 4: 283-285

Professor T. N. ANANTHAKRISHNAN Turns Seventy-One

Professor Taracad Narayanan Ananthakrishnan, fondly known as TNA to several of his friends and colleagues and an eminent Entomologist and Ecologist of India will be completing seventy on 15 December 1995. We, his research students, salute him on the occasion as he radiates dedication and seriousness of purpose to us.

Born as the eldest child to T. K. Narayanan and Annapurani in 1925, he had his early education in St. Aloysius School (Mangalore) and Victoria College (Palakkad). He qualified for his BSc (Hons) degree in Zoology from the Madras Christian College (Madras) in 1946; he won the prestigious Buckie Prize, which was also won by the celebrated entomologist, Dr. T. V. Ramakrishna several years earlier. For a year he was Demonstrator in Zoology in Madras Christian College. Probably pleased with his youthful enthusiasm, in 1948, Principal A. J. Boyd, one of the legendary names of Madras Christian College recommended him to Rev. Jerome D' Souza S. J, another distinguished educationalist of India and Principal of Loyola College (Madras), for appointment as Lecturer. I am not sure if Professor Ananthakrishnan ever dreamt at this point that his link with Loyola College will become a lifelong relationship! Single-handedly, he built and developed the Department. Museum galleries housing extraordinary specimens of insects, birds, reptiles, and mammals stand in testimony to his toil and labour even today.

During this period, he was inspired by Dr. T. V. Ramakrishna who was spending his retired life in the village from which Professor Ananthakrishnan hailed. Dr. Ramakrishna introduced him to thrips and encouraged him to work on this poorly-studied insects. Incidentally, Dr. Ramakrishna also introduced him to Professor M. S. Mani who was then making a powerful impact on the study of Indian insects. Besides his heavy teaching load, Professor Ananthakrishnan took to thrips seriously and his a-little-more-than-decade-long studies enabled him to get his PhD degree from the University of Madras. National and international recognitions began to flow. He won a major PL-480 supported research grant to study the taxonomy, biology, and ecology of Indian thrips. This resulted in the well-received monograph 'Indian Thysanoptera' published by the Council of Scientific and Industrial Research and also the award of Doctor of Science degree by the University of Madras. In the next fifteen years, he was intensely supported by American grants, providing opportunities to him to discover several new species of thrips, their predators and parasites, and develop a better understanding of their ecological relationship with plants in both natural and man-made ecosystems. With the transgovernmental support, he established the Entomology Research Unit, a laboratory meant exclusively for insect study. His bioecological investigations on the thrips infesting weeds along the bunds of crop fields and their migratory patterns from weeds to crops and *vice-versa* made a definitive impact on agricultural entomologists who always believed in controlling pest insects with violent chemicals. Modelling of ecological dynamics of pest thrips of crop plants enabled them to think of alternative and non-violent modes of insect control. In recognition of this work, Professor Ananthakrishnan was given the

Rafi Ahmad Kidwai Award by the Indian Council of Agricultural Research in 1972, only to be followed by many laurels and recognitions. The Indian Academy of Sciences (Bangalore) and Indian National Science Academy (New Delhi) admitted him as a fellow.

In 1978, he went to the Zoological Survey of India (ZSI) to assume charge as its Director, a position occupied by distinguished entomologists like Major M. L. Roonwal. While presiding over the destinies of ZSI, Professor Ananthakrishnan reframed its philosophy of work and reformed its mode of functioning, by giving a distinct ecological orientation from a museum-oriented taxonomic study. He emphasized that an ecosystems approach and population study are but imperative for valid taxonomic decisions. He exemplified it with his own work and his several publications made during his stay there testify this adequately. What is remarkable is that he highlighted the importance of biological diversity and its varied dimensions, at a time when it was never talked about and idealized. His stay at ZSI, though brief, has made indelible changes in research priorities and survey methods. He started five regional research stations in different parts of the country depending on the nature of biological resources those regions prided as heritage materials.

In 1981, he returned to Loyola and the first effort he made was to reset the research goals of his laboratory. The Unit grew into an Institute keeping 'Insect-Plant Interactions' as the Key theme of work. He initiated intense studies on insects of hemipteroid stock and their interactions with plants. Major papers and monographs began to appear in national and international journals, dealing with diverse, but finer aspects of many plant feeding and a few carnivorous Hemiptera, relating to their behaviour and physiology of nutrition and reproduction. The Entomology Research Institute became a stop-over point for many foreign scientists. In 1984, the University Grants Commission (New Delhi) under the COSIST special assistance programme recognized Professor, Ananthakrishnan's brainchild, the Entomology Research Institute, as an important research centre and provided a handsome grant to strengthen the infrastructural facilities. A two-storey building with a large floor area and sophisticated instrumentation facility came up. In February 1986, we, the students of Professor Ananthakrishnan and the Management of the College rejoiced the occasion of its dedication to the Nation along with many topnotch scientists of India and several friends and well-wishers of the Institute. Today, the Institute stands a shoulder above the rest by its consistent publication record, attraction of research grants, national and international recognition, and support to young entomologists of the country.

Further to building an enormous, yet beautifully functional Institute, an admirable quality of Professor Ananthakrishnan is his deep concern for young entomologists and/or biologists enthusiastic to take to entomology. At least a dozen workshops and training programmes have so far been organized by him enabling youth to visit the Institute, get to know senior entomologists, and to become familiar with modern approaches in field methodology and use of appropriate laboratory tools.

In 1988 and 1993, the Institute celebrated the twenty-fifth and thirtieth anniversaries. As usual, the celebrations included academic meetings involving professionals and the publication of critically-edited research books. Today, under his dynamic leadership, the Institute is poised towards the molecular biological study of insect-plant interactions.

We, his graduate students, bow before his greatness. We want to absorb his good traits which are numerous. His commitment to purpose is something all of us adore. A thoroughly self-made person, he created the Entomology Research Institute which enjoys global recognition today. It is not an exaggeration if I say that only his singular efforts have resulted in building such a self-contained facility and a comparison for it will be hard to find for the next few decades!

In a note of tribute like this, my description can be endless because he is a personality of

high achievements; for example, a little more than five hundred research papers and nearly two dozens of reference books and monographs, Jawaharlal Nehru Fellowship, INSA Senior Scientist Award, CSIR Emeritus Scientist Award, and Pitambar Pant Environment Fellowship, Fellowship in professional societies like the Indian Academy of Agricultural Sciences and Institute of Ecology, and Visiting Professor in several British and American Universities are some of his many attainments. On behalf of all my colleagues in the Institute and his past and present research students, I avail this opportunity to pay my respects to this great Indian biologist and wish him very many happy returns of the day and many years of productive entomological and ecological studies. Schöne Geburtstag, Sir!

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ENTOMON 1995 VOL. 20 NO. 3 & 4: 287

BOOK REVIEW

INDIAN FRUIT FLIES

By V. C. Kapoor

Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi
Price Rs. 325.00
pp. 388

Fruit flies are very serious pests on horticultural crops and vegetables. The damage caused by them even crosses 50% in certain cases like mangoes and gourds and volumes of information on the distribution in space and taxonomy of fruit flies are available scattered. The present book "Indian fruit flies" is a methodical collection of such literature. The author vividly describes the taxonomy and distribution of fruit flies. The drawings are self explanatory and serve as authentic guide for the taxonomic identification of fruit flies. Detailed taxonomic key describing diagnostic characters presented in the book will be closely followed by students and researchers in Entomology. The Indian students of Entomology are mainly in agricultural Universities and other universities of applied sciences. The present book is a powerful tool for learning and research in the subject. The arrangement of the topics and other treatment of the subject matter embraces all facets of scientific information. The main aim of this book is bringing out both fundamental and advanced information on fruit flies and their habits, habitat and control. It can be very clearly seen, the author has accomplished the said goal. The lucid tersa style used in the description throughout, projects the book to forefront of literature. Unnecessary and complicated details on taxonomy and zoogeography have been carefully eliminated without loss of any basic information. The subject matter presented gives balanced exposition of the study of fruit flies. The book serves both as a text and reference to students of all levels and researchers. Control of fruit flies have been attempted to in many ways. The basic information on the modern methods of control of fruit flies using lures and traps and also natural enemies have also been concisely and clearly described in the book.

A. Visalakshi

Announcement

A National Symposium on Technological Advancement in Rice Production is being organized by the co-sponsorship of Indian Council of Agricultural Research and Kerala Agricultural University during the 2nd week of May 1996 at RARS, Pattambi. All rice workers of International and National level are requested to participate in the deliberations. Concurrent Sessions are arranged for all the major disciplines in Agricultural Science to make the programme a grand success. The related Circulars will follow. For further details, all are requested to contact in the following address.

Dr. N. Krishnan Nair
Associate Director of Research
Regional Agricultural Research Station
Mele Pattambi - 679306, Palakkad District, Kerala

Recent Advances in Insect Endocrine Research

D. Muraleedharan
Mariamma Jacob
Editors

With lapse of time, the level of information gathered through ingenious research investigations on different scientific disciplines especially that on biological science is really astonishing. About two-three decades before insect endocrine research has been viewed at the cellular or subcellular level. But now we have started looking to view and try to understand insect endocrinology at the molecular level; thanks to the marvellous advances made in developing newer analytical techniques and biotechnological and molecular biological approaches attained in biological science. This has actually revolutionised our whole approach and attitude to look biological processes from a different angle. This volume particularly stresses to review the present status of our information available on different aspects of Molecular Insect Endocrinology through 5 different meticulously written Chapters highlighting the major advances made in the field in recent years. Also the last concluding Chapter convincingly instructs us how best we could use the information gathered through these molecular insect endocrinological research investigations towards developing innovative and newer insect pest management strategies. This volume shall be of immense use both to the students as well as serious researchers of Insect Neuroendocrinology of the immediate future.

The Chapters covered are on: Neuropeptide Diversity in Insect Nervous System (Dick R. Nässel, Sweden) ❑ Molecular Chemistry of Insect (D. B. Tembhare, India) ❑ Endocrines in Insect Embryonic Development (Mariamma Jacob, India) ❑ Neuroendocrinology of Insect Gut (D. Muraleedharan, India) ❑ Endocrine-Based Opportunities for Insect Pest Control (F. Couillaud and L. Peypelut, France).

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Contents of Volume No. 20

Vol. 20 **March 1995** **No. 1**

Vol. 20 **June 1995** **No. 2**

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AUTHOR INDEX

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